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Cell, Vol. 102, 543–548, September 1, 2000, Copyright ©2000 by Cell Press

BIRinging Chromosomes through Cell Division—And Survivin' the Experience

Minireview

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The replication of cells and successful maintenance of cell lineages depend on at least two things. First, the cell must successfully replicate its genetic material and faithfully pass an intact copy of the genome to its progeny at cell division. Second, at least one of the two resulting cells must survive to carry the genome on to the next generation. Tumor cells are particularly adroit at the process of cell replication, out-performing their normal counterparts at the expense of the host organism. However, cancer cells are also known for their genetic instability, and aneuploidy can result when chromosome segregation occurs erroneously. Fortunately, checkpoints that link apoptosis to defects in cell division provide some measure of protection, but dysregulation of apoptosis can create a fertile soil in which genetically unstable cells can thrive.

A family of proteins containing a zinc binding fold, termed the BIR domain, has recently been implicated in chromosome segregation and cytokinesis. Some members of this family also regulate apoptosis. The mammalian prototype of this family is Survivin, an ~16 kDa protein that contains a BIR domain, followed by a long α-helical region important for its targeting to the mitotic-spindle, spindle midbody, and related structures (Altieri and Marchisio, 1999). Yeast, including budding (*S. cerevisiae*) and fission types (*S. pombe*), nematodes (*C. elegans*), and flies (*Drosophila*) contain apparent Survivin orthologs and/or homologs, based on sequence comparisons and (where tested to date) similarities in function with respect to chromosome segregation and cytokinesis. Each of these proteins contains one or two copies of a BIR domain, along with variable additional domains located C-terminal to the BIRs (Figure 1). Besides cell division effects, several BIR-containing proteins serve an entirely different purpose as apoptosis suppressors (Deveraux and Reed, 1999) and some such as Survivin have been proposed to do both. Here we review recent findings concerning mechanisms of BIR family proteins, focusing on Survivin and its homologs in lower organisms.

Survivin and Cell Survival

Before Survivin's discovery, BIR domains had only been recognized in members of the IAP family of apoptosis-inhibiting proteins. In fact, the term BIR (*baculovirus iap repeat*) derives from the original discovery of such antiapoptotic genes in the genomes of baculoviruses, where they help maintain host cell survival long enough to permit virus replication (Miller, 1999). Indeed, overexpression of Survivin delays apoptosis induced by various stimuli, whereas antisense-mediated reductions in Survivin sensitize cells to apoptotic stimuli. Survivin overexpression can also suppress apoptosis even in G_0 arrested cells, thus separating its cytoprotective function from cell cycle effects. Several human and fly IAPs bind directly to and suppress caspase family cell death proteases, explaining their antiapoptotic activity (Deveraux and Reed, 1999). Survivin also reportedly binds certain caspases in vitro, though the interactions are far less convincing than those observed for other IAPs. Furthermore, purified recombinant Survivin protein lacks the ability to inhibit caspase-3, unlike other human IAPs (Verdecia et al., 2000). Nevertheless, Survivin reportedly coassociates with caspase-3 in the vicinity of centrosomes during mitosis, and is required for suppression of caspase-mediated cleavage of centrosome-associated p21waf1 (Li et al., 1999)—consistent with the hypothesis that Survivin may be a caspase inhibitor at least in some spatial contexts. However, Survivin also binds quantitatively to an IAP-inhibiting protein, Smac/Diablo, at least in vitro (Du et al., 2000), raising the possibility that it might suppress caspases indirectly by freeing other IAP family proteins from constraints of this protein.

Insights from Survivin Homologs in Yeast and Worms

Homozygous disruption of the only BIR family gene of *S. cerevisiae* (*BIR1*) results in severe defects in tetrad formation by diploid cells when induced to sporulate, suggesting problems with meiosis (Uren et al., 1999). Although haploid cells lacking *BIR1* can be recovered, they die after a few mitotic divisions. Targeted disruption of *BIR1* in haploid yeast does not prevent vegetative growth, but the cells do show a defect in chromosome segregation (Yoon and Carbon, 1999). Furthermore, Δ*bir1*/Δ*bir1* diploids have abnormal DNA content (Li et al., 2000), accumulating extra genetic material, consistent with a chromosome segregation defect and aneuploidy. Haploid Δ*bir1* budding yeast exhibit abnormal morphology, and this can be rescued by plasmid-derived expression of *BIR1* but not mammalian Survivin (Li et al., 2000), suggesting perhaps an important role for the unique C-terminal domain of yeast Bir1p (Figure 1). Indeed, the C-terminal domain is responsible for Bir1p association with the spindle apparatus of anaphase cells (Uren et al., 1999).

Similar phenotypes have been observed for the BIR family proteins of fission yeast, *C. elegans*, and mammalian cells. For example, targeted disruption of the *bir1* gene of *S. pombe* results in mutant cells with a "cut phenotype," characteristic of cell cycle mutants with problems in completing mitosis or anaphase (Uren et al., 1999). Tubulin staining of these *bir1* mutants reveals shortened mitotic spindles, implying a problem with progression from metaphase to anaphase. Likewise, suppression of *bir-1* expression in *C. elegans* embryos by RNA interference (RNAi) results in a single giant multinucleated cell due to a complete failure of cytokinesis (Fraser et al., 1999). Also, *bir-1*(RNAi) fertilized oocytes and embryos exhibit problems with chromosome condensation, metaphase alignment, and anaphase segregation of chromosomes as well as defects in spindle midzone formation (Speliotes et al., 2000). Finally, suppression of human Survivin expression by antisense

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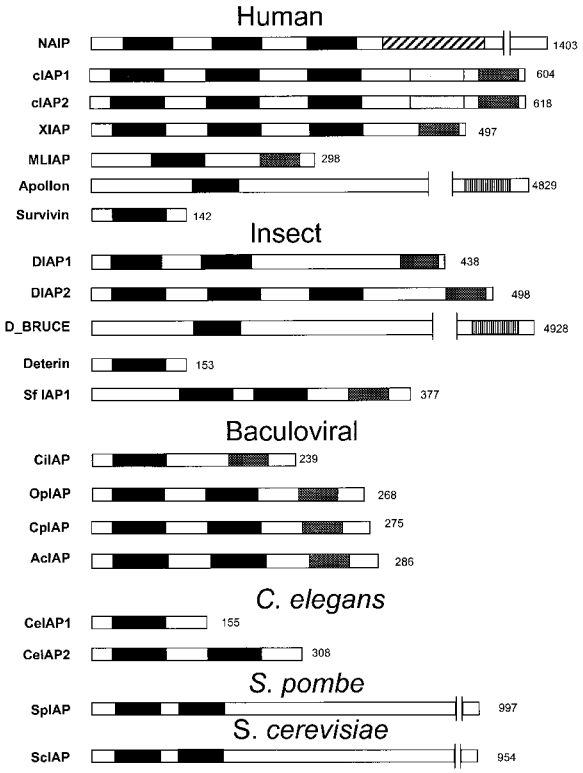

Figure 1. BIR Family Proteins

Schematic of the BIR family proteins, showing presence of BIR domains, as well as CARD (caspase-associated recruitment domain), putative NB (nucleotide binding), Ub (ubiquitin-conjugating), and RING domains.

methods or ectopic expression of a *trans*-dominant inhibitory mutant of Survivin in human cells results in polyploidy, multinucleated cells, and abnormal mitotic spindles causing multipolar mitoses (Li et al., 1999).

Surprises in the 3D-Structure of Survivin

Immunolocalization has provided evidence that mammalian Survivin associates with mitotic spindle microtubules, centrosomes or pericentrosomal microtubules, and the cytokinetic remnant—a filamentous structure representing the last bit of connecting material joining two daughter cells at the end of telophase (Li et al., 1999). The C-terminal (non-BIR) domain of Survivin is required for its colocalization with mitotic spindles and centrosomes and its ability to bind polymerized microtubules in vitro. Mutations in the BIR of Survivin do not interfere with targeting, but can result in dominant-negative effects, inducing polyploidy and apoptosis when overexpressed in mammalian cells. Clues to the possible dominant-inhibitory mechanism of Survivin mutants have come recently from X-ray crystallographic analysis of the structures of human (hu) and mouse (mu) Survivin proteins, revealing for the first time that Survivin forms dimers (Chantalat et al., 2000; Muchmore et al., 2000; Verdecia et al., 2000). Dimerization of huSurvivin is mediated principally by hydrophobic interactions involving to some extent the BIRs but particularly residues located between the BIR and C-terminal long α helix region, which are conserved in the human, mouse, and fly Survivin proteins but not found in the BIRs of XIAP, cIAP1, or other caspase-inhibiting IAPs. However, the structural details of Survivin dimerization are not without controversy, as one of three alternative interpretations of the crystallographic data obtained for the mouse protein suggested that an unusual zinc-chelation mechanism mediates interactions of the monomers, involving covalent interactions of the metal with a histidine and (unexpectedly) a glutamate residue (Chantalat et al., 2000). Furthermore, mutagenesis of these zinc binding residues disrupted dimerization and altered function of muSurvivin. However, one of the possible muSurvivin dimer structures agrees well with the reported huSurvivin dimer structures. Additional mutagenesis experiments should yield consensus about the dimerization interface.

The huSurvivin monomer consists of a zinc binding fold (a three-stranded antiparallel β sheet surrounded by four short α helices) highly similar to the BIR2 domain of XIAP, followed by a long amphipathic α helix that extends outward, giving a "bow tie" or "butterfly" appearance in the dimer. Intriguingly, extended α helices are common in tubulin binding proteins, consistent with

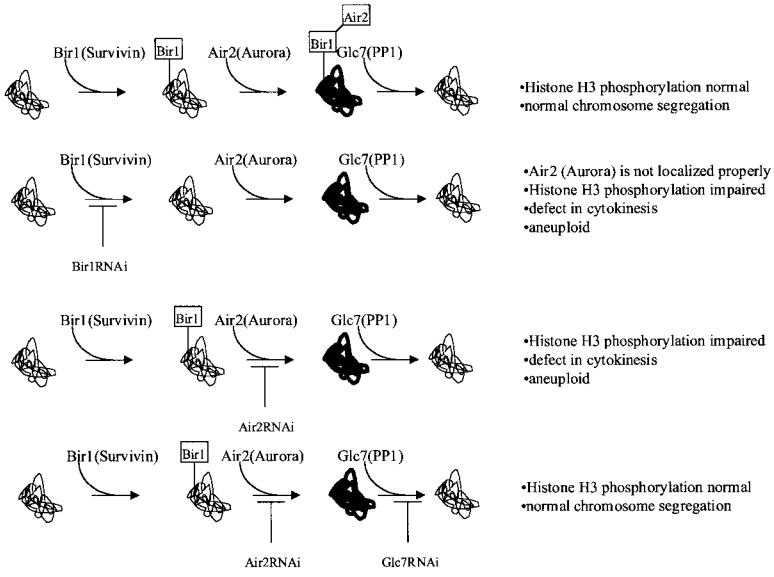

Figure 2. Model of the Potential Bir1/Air2 Pathway in *C. elegans*

Bir1 is localized to chromosomes by an as yet unknown mechanism. The binding of Bir1 to chromosomes is required for the subsequent localization of Air2 to the metaphase chromosomes. Once localized to the chromosomes, Air2 phosphorylates histone H3 on serine 10. Glc7 then dephosphorylates histone H3. It is not yet clear which mitotic process this pathway is regulating. One possibility is it is responsible for the maintenance of phosphorylation of histone H3 either globally or locally during the metaphase-to-anaphase transition to ensure the daughter chromosomes are properly segregated to the poles. The fact that Ce*glc-7b*(RNAi) embryos do not develop past the one-cell stage despite restoration of histone H3 phosphorylation suggests that Air2 has substrates other than histone H3.

Survivin's reported interaction with stabilized microtubules, but precisely what proteins interact directly with this domain is unknown. The BIR of Survivin also has a striking acidic surface not seen in the structures of caspase-inhibiting IAPs. Although Survivin lacks a region corresponding to the BIR2-proximal segment of XIAP critical for caspase-3 inhibition (Sun et al., 1999), its BIR domain bears some interesting resemblance to the caspase-9 binding domain (CARD) of Apaf-1 (Chantalat et al., 2000).

BIR-Containing Proteins Interact with Kinetochores

So, why is Survivin/Bir1 important for chromosome segregation and cytokinesis? The first clue came from a two-hybrid screen for Ndc10p-interacting proteins, revealing an association with Bir1p (Yoon and Carbon, 1999). Ndc10p is a component of the yeast kinetochore. The kinetochore is a centromere-associated multiprotein structure that hooks condensed chromosomes to the ends of microtubules of the spindle apparatus. Bir1p also binds at least weakly to Skp1p, which is another essential component of the kinetochore. Genetic evidence further supports an important functional interaction of Bir1p with kinetochore components in yeast. Analysis of diploids carrying heterozygous (+/−) *bir1*Δ mutations and heterozygous mutations in genes encoding yeast kinetochore proteins revealed synthetic lethal interactions with *cbf1*Δ, *ctf19*Δ, and *skp1–4* mutants. By contrast, combining heterozygous mutations of *bir1*Δ with mutations in mitotic spindle checkpoint mutants (*mad1, mad2, mad3, bub1, bub2, bub3*) did not result in synthetic lethal interactions, implying that Bir1p operates on a distinct component of the chromosome segregation machinery. Further evidence of genetic interactions between yeast Bir1 and the kinetochore has been obtained by overexpression studies, revealing that overexpression of *NDC10* completely rescues the chromosome-loss phenotype of *bir1*Δ cells and overexpression of *BIR1* increases chromosome segregation fidelity in *skp1–4* mutants (Yoon and Carbon, 1999).

Taken together, these observations raise the possibility that the chromosome segregation defects seen in Bir1p-deficient yeast may reflect a problem in kinetochore assembly or function. However, one cannot necessarily surmise that the same protein interactions are relevant for the Bir1/Survivin homologs of animal cells, as the nonconserved C-terminal domain of yeast Bir1p (rather than the BIR domains) is responsible for interaction with Ndc10p. In addition, this C-terminal fragment is as efficient as full-length Bir1p at rescuing chromosome segregation defects in *bir1*Δ null and *skp1–4* yeast.

Kinases Linked to BIR Proteins

At present it is unknown if Bir1/Survivin proteins are phosphorylated but the Bir1p-interacting protein Ndc10p is. The phosphorylation state of Ndc10p in yeast is likely to be regulated by the protein kinase Ipl1p and the type-I protein phosphatase (PPase) Glc7p (Biggins et al., 1999). Ipl1p is a serine/threonine kinase that was originally isolated in genetic screens for mutants that increase ploidy (Bischoff and Plowman, 1999). It is a member of the Aurora-Ipl1-like family of kinases that have been implicated in control of chromosome segregation and cytokinesis. Family members share a conserved C-terminal kinase domain but are more variable in their N-terminal regions. Humans have at least three aurora family kinases (Aur1, 2, 3), whereas two members are found in *C. elegans* (Air1, Air2) and *Drosophila* and one in *S. cerevisiae* and *S. pombe*. Aurora/Ipl1-like kinases are produced and become activated specifically during mitosis. Aur1 localizes to the midzone of anaphase cells and postmitotic bridge of telophase cells. Overexpression of catalytically inactive Aur1 mutant results in multinucleated cells due to cleavage furrow failure, thus resembling the phenotype seen when Survivin expression is ablated by antisense. Aur2, which becomes active before Aur1, is associated with centrosomes of interphase cells and the mitotic spindle from prophase through telophase. Aur2-deficient human cells arrest in late prophase prior to the alignment of the chromosomes on the metaphase plate (Bischoff and Plowman, 1999), which is not typical of Survivin deficiency. However, it is interesting that the localization of Survivin partially overlaps with that of Aur1 and Aur2. Very similar roles for the homologous kinases have been documented in *C. elegans*, showing that worm Air1 and Air2 are the orthologs of human Aur2 and Aur1, respectively (Schumacher et al., 1998; Bischoff and Plowman, 1999).

In yeast, a delicate balance between the opposing effects of aurora-kinase Ipl1p and PPase Glc7p is required for proper chromosome segregation: partial loss-of-function mutations of *ipl1* are complemented by mutations in Glc7 (Hsu et al., 2000). Ndc10p is hyperphosphorylated in *glc7* mutant strains, and is an efficient substrate of Ipl1 in vitro, suggesting that this Bir1p binding protein represents one of the relevant substrates of this kinase/PPase pair. Ipl1p and Glc7p have also recently been implicated in control of phosphorylation of histone H3 on serine 10 (Hsu et al., 2000). H3 phosphorylation at Ser10 is a conserved feature of mitosis and meiosis from fungi to humans, with H3 phosphorylation associated with condensed chromosomes from prophase to telophase. In *Tetrahymena*, a Ser10Ala mutation leads to abnormal chromosome segregation and marked chromosome loss during mitosis and meiosis (Wei et al., 1999). Some mutant alleles of *glc7* suppress mutants of *ipl1*, restoring growth and in vivo phosphorylation of H3 (Hsu et al., 2000). Glc7 also dephosphorylates H3 in vitro, and Ipl1p phosphorylates H3 at Ser10 in vitro, suggesting a direct effect on H3.

Analogous to yeast, RNAi-mediated inhibition of *AIR-2* causes defective histone H3 phosphorylation, at least as measured by staining with antibodies that detect phosphorylation of H3 serine 10. By contrast, H3 phosphorylation is normal in *air-1*(RNAi) embryos (Hsu et al., 2000). The *C. elegans* genome contains two potential Glc7 orthologs, Ce*GLC-7a* and *7b* (Hsu et al., 2000). RNAi-mediated suppression in worms of either of these restores H3 phosphorylation in *air-2* (RNAi) mature oocytes and embryos. Interestingly, Ce*glc-7a*(RNAi)/*air-2* (RNAi) embryos also develop beyond the 1-cell stage, whereas Ce*glc-7b*(RNAi)/*air-2*(RNAi) embryos do not, despite restoration of H3 phosphorylation. This observation implies that Air2 has other substrates besides H3 which are important for cytokinesis, consistent with the localization of Air2 not only to condensed chromosomes but also to midbody microtubules during embryonic cell divisions in the worm (Schumacher et al., 1998).

As in real estate, the three most important considerations in kinase/phosphatase actions are location, location, and location. RNAi methods in *C. elegans* showed recently that Bir1 is required for targeting of Air2 to metaphase chromosomes (Speliotes et al., 2000). As RNAi-mediated suppression of Air2 does not impair Bir1 localization, the supposition is that Bir1 interacts with

structures on condensed chromosomes and then recruits Air2 to substrate targets. Indeed, H3 phosphorylation of metaphase chromosomes and the localization of the kinetochore protein, HCP-1, is reduced in both *air-2*(RNAi) and *bir-1*(RNAi) embryos (Speliotes et al., 2000). Additional circumstantial evidence linking Bir1 and Air2 in the worm includes the observation that RNAi-mediated suppression of *BIR-1* or *AIR-1* expression results in indistinguishable defects in chromosome segregation and cytokinesis (Schumacher et al., 1998; Fraser et al., 1999; Speliotes et al., 2000). For example, the phenotypes of *bir-1*(RNAi) and *air-2*(RNAi) embryos are strikingly similar, with fertilized oocytes failing to progress beyond the 1-cell stage, accumulating nuclei without undergoing cell divisions (Schumacher et al., 1998; Fraser et al., 1999; Speliotes et al., 2000). Also, comparisons by immunofluorescence microscopy of Air2 and Bir1 on metaphase chromosomes and the spindle apparatus of cells in mitosis and anaphase suggest identical spatial and temporal localization. Unfortunately, direct evidence that Bir1 binds Air2 is lacking at this time, and several additional proteins might operate between Bir1 and Air2 within a common pathway.

Implications of Genetic Instability and Cancer

While studies in yeast and *C. elegans* are beginning to paint a picture in which functional interactions of Bir1 proteins with aurora family kinases play a critical role in chromosome dynamics, nothing is known presently about the relevance of these kinases to mammalian Survivin. Human Survivin partially rescues *bir-1*(RNAi) embryos, allowing ~20 cell divisions (Fraser et al., 1999; Speliotes et al., 2000), but whether this correlates with restoration of Air2 targeting to chromosomes and phosphorylation of H3 remains unexplored.

It is intriguing, nevertheless, that the genes encoding Aurora kinases and Survivin share many similarities, including cell cycle–dependent expression during G_2/M, aberrant overexpression in most human cancers, and association with poor patient prognosis (Bischoff and Plowman, 1999; Deveraux and Reed, 1999). In higher eukaryotes, when cells become aneuploid they usually undergo apoptosis, but this is suppressed in tumor cells. Thus, the genetic connection in lower organisms between Aurora family kinases and Bir/Survivin family proteins is compelling and could lead to an explanation of how aneuploid tumor cells avoid apoptosis. If so, then the path of least resistance in terms of drug development for cancer would presumably involve inhibitors of the kinases rather than Survivin—to the extent that Aurora kinases and Survivin perform superimposable functions. However, do they operate entirely within a common pathway or does Survivin have a split personality as a regulator of both apoptosis and cytokinesis? One way to approach this is by comparing Survivin to the caspase-inhibiting IAPs. Sequence analysis of BIR domains indicates that Survivin fits better into the subgroup of BIR family proteins that contains yeast and *C. elegans* homologs than the caspase-inhibiting mammalian and insect IAPs (Deveraux and Reed, 1999). Clearly, the Survivin homologs of budding and fission yeast cannot be caspase inhibitors, as their genomes lack caspase genes. Also, no genetic interactions were observed between *bir-1*(RNAi) or *BIR-1* overexpression and cell death gene mutants (ced-3, ced-4) in worms (Fraser et al., 1999; Speliotes et al., 2000). However, there are differences between the death machinery of worms and other animal species, such as an absence of Bax homologs and variations in the mechanisms of Bcl-2 and CED-4/Apaf-1 homologs compared to flies and mammals. In this regard, *Drosophila* contains a Survivin homolog (*deterin*) that reportedly blocks cell death and is required for insect cell survival in culture (Jones et al., 2000), yet its BIR (like Survivin's) is more similar to the BIR proteins of yeast and *C. elegans* than the caspase binding BIRs of fly IAPs. Furthermore, in yeast, it is the C-terminal unique domain (not the BIRs) that correlates with cell division effects (Yoon and Carbon, 1999). So, perhaps the Bir1 proteins of yeast and worms are not true orthologs of fly Deterin and mammalian Survivin, but rather homologs from which Survivin emerged in higher eukaryotes, having gained a second antiapoptotic function that links suppression of apoptosis to chromosome segregation and cytokinesis, thereby establishing a checkpoint mechanism for this vulnerable period of the cell cycle. Time will tell.

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