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Genome Stress Response in Early Development

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Abstract

Cells with irreparable genomic damage pose a problem for development and must be eliminated to prevent disease. Reporting in *Developmental Cell*, Iampietro et al. (2014) describe a mechanism in *Drosophila* that removes damaged nucleifrom syncytial blastodermembryos via DNA damage checkpoint kinase-mediated retention of mRNAs within the nucleus.

An essential feature of animal development and tissue homeostasis is the repair or elimination of cells with genome damage that arise spontaneously (e.g. through errors in DNA replication or chromosome segregation) or after exposure to exogenous genotoxic insults (e.g. irradiation). In response to such stresses, cells activate an evolutionarily conserved kinase cascade involving the Chk1 and Chk2 checkpoint kinases, which in turn activate p53 to induce the transcription of genes that result in either G1 cell cycle arrest and DNA repair or apoptosis, depending on the cell type and physiological circumstance. In the early embryos of some organisms, however, activation of these checkpoint pathways does not cause cell cycle arrest or apoptosis (Su, 2010), raising the question of how these embryos avoid incorporating cells with genomic damage into developing tissues. Evolution has of course crafted an answer, and in this issue of *Developmental Cell*, Iampietro et al. (2014) show using a beautiful combination of cell biology, biochemistry, and genetics that the elimination of such damaged genomes from developing *Drosophila* embryos involves a Chk2-dependent pathway that results in retention of specific mRNAs within the nucleus.

During animal development, a dramatic expansion in cell number often occurs immediately after fertilization. In *Drosophila*, this is achieved through rapid (i.e. as short as 8 minutes) nuclear division cycles that lack G1 and G2 phases and occur in a common cytoplasm. These syncytial cycles give rise to a monolayer of ~6000 cortical nuclei that cellularize to

form the blastoderm. The descendents of these 6000 blastoderm cells give rise to every tissue in the animal. Thus, nuclei containing genomic aberrations resulting from DNA damageor chromosome segregation errors during mitosis could negatively impact development if not eliminated prior to blastoderm formation. While p53-mediated apoptosis eliminates cells with damaged genomes in later stages of development (e.g. post-embryonic), nuclei with genomic damage in syncytial embryos are instead actively translocated into the yolk in the interior of the embryo, thereby removing them from the blastoderm and preventing them from subsequently contributing to developing tissues (Sullivan et al., 1993). As is the *Drosophila* custom, this process has been cleverly coined “nuclear fallout.”

Nuclear fallout is triggered by a variety of stimuli, but the mechanisms have not been fully elucidated. In the final syncytial cycles just prior to cellular blastoderm formation, Chk1 function is required to delay initiation of key mitotic events like nuclear envelope breakdown, chromosome condensation, and possibly anaphase onset until chromosomes are fully replicated (Sibon et al., 1997; Yu et al., 2000). Consequently, Chk1 mutants display extensive anaphase bridging, resulting in nuclear fallout. Other insults to genome integrity, such as addition of drugs that inhibit DNA replication or cause double strand breaks, also result in chromosome segregation errors during mitosis and subsequent fallout of damaged daughter nuclei (Takada et al., 2003). In addition, although there is no cytokinesis during syncytial nuclear divisions, transient, actin-rich invaginations of the plasma membrane, called pseudocleavage furrows, form between nuclei during mitosis. This process effectively isolates each mitotic spindle from its neighbors in the common egg cytoplasm, ensuring that each daughter nucleus remains diploid and preventing potentially deleterious microtubule interactions between adjacent spindles. Disruptions to the actin cytoskeleton that prevent normal pseudocleavage formation also cause aberrant syncytial division and nuclear fallout (Riggs et al., 2003).

A clue to the mechanism of nuclear fallout came with the observation that centrosomes, which normally closely associate with syncytial nuclei, are left at the cortex when damaged nuclei move to the interior of the embryo (Sullivan et al., 1993). Disruption of centrosome integrity and function in response to DNA damage and chromosome segregation errors accompanies nuclear fallout (Sibon et al., 2000).Building on this observation, it was shown that Chk2 localizes to and inactivates centrosomes resulting in nuclear fallout (Takada et al., 2003). Centrosomes play multiple roles in syncytial embryos, including formation of the bipolar spindle and coordinating pseudocleavage furrow formation, and mutations that inhibit centrosome separation during mitosis cause chromosome segregation errors and trigger Chk2-dependent nuclear fallout (Poulton et al., 2013). How Chk2 functions in nuclear fallout is not fully known, but identifyingChk2 substrates and understanding how phosphorylation affects their function should shed light on the mechanism.

This is where Iampietro et al. (2014) come in. They noticed when using in situ hybridization to examine the expression of many genes in syncytial embryos that, rather than being exported to the cytoplasm, certain mRNAs accumulate to high levels within nuclei undergoing fallout. Interestingly, these mRNAs encode proteins essential for nuclear division that function in the actin cytoskeleton, centrosomes, and chromatin. The authors

observed a reduction in the concentration of several of these proteins in nuclei undergoing fallout, suggesting that mRNA retention in the nucleus leads to local reductions in translation that could disrupt mitosis and contribute to nuclear fallout. One class of retained mRNAs was those encoding the replication-dependent histones. These mRNAs are unique because they are not polyadenylated and instead end in a highly conserved stem loop structure that is formed through a special 3' end processing reaction (Marzluff et al., 2008). The authors show that the histone mRNA 3' UTR and stem loop is sufficient to confer nuclear retention to a heterologous gene. The histone mRNA 3'end stem loop binds a protein called "stem loop binding protein" (SLBP) that participates in all aspects of histone mRNA metabolism, including export from the nucleus and translation of the mRNA (Marzluff et al., 2008).In the current paper, the authors convincingly show using a comprehensive collection of biochemical, cell biological, and genetic experiments that Chk2 phosphorylates SLBP on threonine 118, leading to degradation of SLBP and nuclear retention of histone mRNA.*kuk* mRNA, which is required for mitosis, is also retained in the nucleus in a Chk2-dependent manner, and the 3' UTR from this poly A+ mRNA also promotes nuclear retention. Thus, Chk2 may contribute to nuclear fallout by coordinating the retention of different mRNAs via phosphorylation of a specific set of 3'UTR binding proteins.

One important question these data raise is whether nuclear retention of mRNA is a cause or a consequence of nuclear fallout. Iampietro et al. (2014) show that Leptomycin B, which inhibits CRM1-mediated nuclear export of some mRNAs, and mutation of *uap56*, which encodes an mRNA export protein, both induce nuclear fallout. In addition, nuclear fallout increases in embryos lacking zygotic production of histones because of homozygous deletion of the histone locus (these embryos use maternal stores of histones to reach the blastoderm stage). This result is consistent with a model whereby phosphorylation of SLBP by Chk2 in one nucleus triggers degradation of SLBP and retention of histone mRNA in that nucleus, stopping zygotic histone protein expression in the local area of that nucleus. The result is production of less histone protein for that nucleus and a failure to complete DNA replication, leading to DNA damage and nuclear fallout. It is possible that Chk2-mediated nuclear mRNA retention reinforces a commitment to the nuclear fallout pathway by amplifying an initial DNA damage signal through the loss of histone biosynthesis or other factors required for mitosis, resulting in a more global defect in syncytial nuclear division and robust nuclear fallout. However, despite the tight correlation between nuclear mRNA retention and fallout, more work is necessary to directly prove that localized reduction in protein accumulation due to nuclear retention of a specific mRNA causes nuclear fallout. Nevertheless, the authors' interesting observations are sure to stimulate future investigations and discoveries of the different mechanisms organisms use to protect development and homeostasis by eliminating cells with irreparably damaged genomes.

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