Dispatches

Nuclear Morphology: When Round Kernels Do the Charleston

New studies in *Drosophila* have identified a novel nuclear envelope protein with a farnesyl moiety, termed Kugelkern/Charleston, that helps regulate the size, shape and position of cellular blastoderm nuclei.

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The nucleus is the most distinctive feature of eukaryotic cells in terms of both shape and function. It hosts the chromosomes and harbors many of the fundamental cellular activities. The nuclear envelope encompasses the nucleus and is composed of outer and inner nuclear membranes, the nuclear lamina and nuclear pore complexes (NPCs). The nuclear lamina is composed of lamins and lamin associated proteins. It primarily underlies the inner nuclear membrane and plays a key role in determining nuclear structure and is involved in most of the nuclear functions [1]. Consequently, mutations in lamina proteins cause various diseases — collectively known as laminopathies — that affect muscle, adipose, bone, nerve and skin cells and range from muscular dystrophies to accelerated aging [2]. An aberrant nuclear envelope and abnormal nuclear morphology characterize the diseased cells [3,4].

In the early embryonic development of *Drosophila*, during the transition from syncytial to cellular blastoderm, the aligned nuclei are positioned apically near the cortex and synchronously elongate towards the basal plane of the newly forming epithelium [5]. The homogeneous appearance of the chromatin, which marks the syncytial blastoderm, is lost and chromocenters — sites of integration of pericentric heterochromatin — appear at the apical side of the nuclei. These changes in chromatin coincide with the strong increase of zygotic transcription at this stage. During this process, the nuclear envelope doubles its surface area, lobulates and forms indents along the apicobasal axis that accommodate the surrounding microtubules and subsequently help stabilize nuclear columnar structure towards gastrulation.

An exciting piece is added to the growing puzzle of the nuclear envelope through two recent papers by Brandt *et al*. [5], in this issue of *Current Biology*, and by Pilot *et al*. [6]. Both groups used data obtained from comparative developmental microarray analyses to search for genes that when knocked down show defects in the formation of the cellular blastoderm. One of the most intriguing genes identified in this screen was *kugelkern* (German for ‘round kernel’), also known as *charleston* (*kuk*/*char*). In embryos lacking *kuk*/*char* activity, nuclei loose their elongated wildtype shape and their position during the fast phase of cellularization; they dislodge from the cortex, round up and have a smooth and relaxed nuclear envelope surface with a substantially enlarged cross-section. This disrupts nuclear alignment, yielding ‘dancing nuclei’. Staining embryos with disrupted *kuk*/*char* expression with antibodies directed against Heterochromatin protein 1 (HP1) showed that the distribution of HP1 and the formation of the distinct chromocenter are abnormal, suggesting that they depend on Kuk/Char activity. Ultimately, the aberrant shape and position of *kuk*/*char* nuclei disrupt epithelial organization. Brandt *et al*. [5] also identified a second gene that affects nuclear morphology during cellularization termed *kurzkern* (*kur*) (‘short kernel’).

Downregulation of *kur* inhibits nuclear elongation, but not lobulation, suggesting that nuclear elongation and lobulation are independent processes.

The Kuk/Char protein is localized to the nuclear side of the inner nuclear membrane, where it colocalizes with lamin Dm_0. Three regions in Kuk/Char: a coiled-coil domain, a nuclear localization signal (NLS) and a CaaX motif are each required for this inner nuclear membrane localization. Besides the lamins, Kuk/Char is the only known nuclear protein to have a CaaX motif. This carboxy-terminal motif undergoes three post-translational modifications. First, the cysteine is farnesylated, then the last three residues (aaX) are cleaved off and subsequently the cysteine undergoes methyl esterification [7]. Kuk/Char lacking the CaaX motif or Kuk/Char expressed in the presence of farnesyl-transferase inhibitors, failed to localize to the inner nuclear membrane. In addition, *Drosophila* embryos treated with farnesyl-transferase inhibitors were indistinguishable from *kuk*/*char* RNAi embryos, indicating that Kuk/Char localization at the nuclear envelope is crucial for its function [6]. Interestingly, all three elements (coiled-coil dimerization, NLS and CaaX) characterize lamins as well, albeit Kuk/Char does not require lamins for its nuclear envelope localization [5,6]. Brandt *et al*. [5] further demonstrate that overexpression of Kuk/Char in *Drosophila* embryos, either by mRNA injection or by expression of 6 copies of the gene, causes ruffling and over-lobulation of nuclei during cellularization. Moreover, when expressed in *Xenopus* A6 cells, Kuk/Char localizes exclusively to the nuclear envelope causing it to become much larger and highly lobulated.

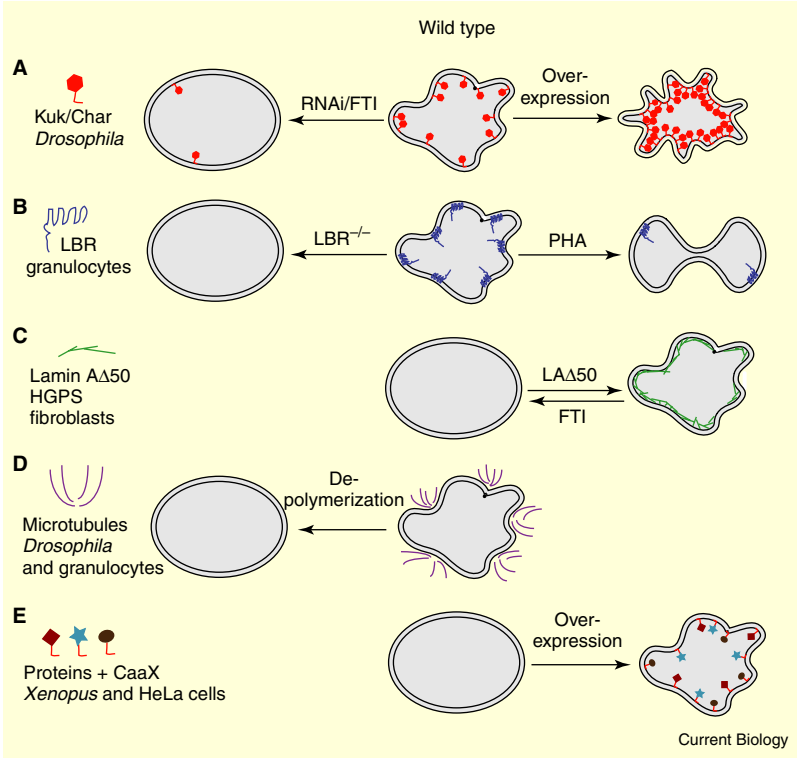

Figure 1. Some of the factors affecting nuclear and nuclear morphology in various experimental systems.

The middle column shows a sketch of the normal morphology of nuclei in a given model system, while left and right columns schematize the aberrant nuclear morphology. (A) Quantitative effect of Kuk/Char on nuclear lobulation in *Drosophila* embryos [5,6]. (B) Loss of lobulation in granulocytes in patients homozygous (left) or heterozygous (right) to mutations in LBR. Heterozygous mutations in LBR cause Pelger Huet Anomaly (PHA), while loss of LBR also cause other phenotypes [20]. (C) Expression of the mutant lamin A (LAΔ50) in HGPS fibroblasts causes nuclear lobulation, which can be reversed by treatment with a farnesyl transferase inhibitor (FTI) [4,15,16]. (D) Microtubules depolymerization causes loss of nuclear lobulation in *Drosophila* embryos and in human granulocytes [11,15,16]. (E) Nuclear envelope localization of proteins containing a CaaX motif causes nuclear lobulation and nuclear membrane proliferation [18,19].

The position of the nucleus within each cell is regulated and nuclear positioning requires the activity of actin, microtubules and microtubule-dependent motors. One way to position nuclei is to mechanically link the nuclear lamina to cytoskeletal elements, perhaps by forming protein–protein complexes that bridge the two nuclear membranes [8]. These links involve novel membrane proteins containing a KASH-domain, which localize uniquely to the outer nuclear membrane, but not the endoplasmatic reticulum [9]. Such proteins include the giant nesprin proteins, which bind either F-actin or cytoplasmic intermediate filaments, and UNC-83, which links nuclei to the microtubule network [10]. The KASH domain proteins 'hold hands' with proteins containing a SUN-domain, which in turn binds the lamin meshwork [9].

While downregulation of Kuk/Char did not affect microtubule organization, depolymerization of microtubules by drugs resulted in phenotypes similar to those observed in Kuk/Char depleted embryos. Pilot *et al*. [6] suggest that Kuk/Char helps link the nucleus to the cytoskeletal filaments, either directly or *via* other protein complexes, perhaps those that include SUN-domain proteins. However, direct proof for this model is missing. By contrast, Brandt *et al*. [5] favor a model in which Kuk/Char is required for nuclear envelope structure, allowing the microtubules to passively sculpture nuclear shape. Support for this model comes from studies showing that, in mammalian granulopoiesis, the integrity of the microtubules system, but not the actin microfilament system, is essential for the nuclear lobulation process [11].

Kuk/Char joins a growing list of nuclear envelope proteins that dramatically affect overall nuclear structure (Figure 1). The mammalian prelamin A protein contains a carboxy-terminal CaaX motif, which undergoes farnesylation. The last 15 amino acids are then cleaved leaving a non-farnesylated mature lamin A. Hutchinson-Gilford Progeria Syndrome (HGPS) is a dominant negative accelerated-aging disease. The most common HGPS mutation is a deletion of 50 amino acids that includes this cleavage site, thus leaving the farnesyl group at the carboxyl terminus of lamin A [12,13]. The nuclei of HGPS fibroblasts in culture become highly lobulated [4]. Applying farnesyl-transferase inhibitors to these fibroblasts reverses the HGPS nuclear blebbing phenotype [14–16], resembling the *kuk/char* depletion effect. Similarly, deficiency of the zinc metalloproteinase, *ZMPSTE24* (*FACE-1*), which is involved in post-translational proteolytic cleavage of the farnesylated prelamin A, probably leads to accumulation of farnesylated prelamin A and to lobulation of nuclei [17]. In line with these observations, ectopic expression of farnesylated proteins targeted to the nuclear envelope causes nuclear lobulation in human HeLa or *Xenopus* A6 cells [18,19]. Several other mutations in lamin A causing muscular dystrophy, lipodystrophy or accelerated aging diseases also show lobulation of nuclei, probably due to the weakening of the nuclear lamina. Similarly, lack of lamin A in mice or human or lack of Ce-lamin in *C. elegans* cause lobulation, suggesting again that lamins play a major role in keeping the nuclear shape (reviewed in [1,2]). Another example of an inner nuclear membrane protein that dramatically affects the overall nuclear structure is lamin B receptor (LBR). It contains 8 transmembrane domains and

interacts with Lamin B, chromatin and HP1. Mutations in LBR cause the Pelger-Huet Anomaly (PHA), which is characterized by loss of nuclear lobulation in granulocytes [20].

The study of the nuclear lamina is becoming more and more exciting, particularly in terms of identifying its roles in regulating the organization and various functions of the nucleus. Different heritable laminopathies are caused by novel mutations in lamina proteins and recently developed drugs, including farnesyl-transferase inhibitors, that ameliorate the cellular phenotypes caused by laminopathies are currently put into clinical trials. Kuk/Char adds an important link to understanding the role of the nuclear lamina in determining nuclear shape, structure and position within the cell, which is relevant for understanding the disease phenotypes. Further work on Kuk/Char and nuclear architecture may provide novel insights into how nuclear structure and position are determined.

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Mitochondrial Protein Import: Convergent Solutions for Receptor Structure

Complex machinery has evolved to recognise and import nuclear-encoded proteins into mitochondria. Recent work now shows that the plant Tom20 mitochondrial protein import receptor has a similar tertiary structure to animal Tom20, although the proteins are evolutionarily distinct, representing an elegant example of convergent evolution.

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Mitochondria are generally accepted to have descended from a eubacterium that was engulfed by an archaebacterial host cell [1,2]. During the evolution of this endosymbiotic relationship, the vast majority of organellar genes were transferred to the nucleus, necessitating an efficient system to import nuclear-encoded mitochondrial proteins into the organelle [2]. All extant mitochondria possess this protein import machinery, consisting of the translocase of the outer mitochondrial membrane (TOM) complex (Figure 1), and two inner mitochondrial membrane complexes [3]. The TOM complex consists of two functionally defined groups of proteins that either form the 'general import pore' or act as receptors that facilitate delivery of the precursor protein to the pore [4].

Two apparent obstacles that had to be overcome in order to allow for