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Auxetic nuclei

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Abstract

The nucleus of naïve mouse embryonic stem cells in transition to differentiation expands when the cells are stretched and contracts when they are compressed. What drives this auxetic phenotype is, however, unclear.

The characterization of both metastable and fully committed embryonic stem cells (ESCs) — pluripotent cells that are able to differentiate into almost all cell types in the body — is highly elusive. Because metastable cells can return to their undifferentiated state or become fully committed to differentiation, these physiological ESC states are crucial to the understanding and control of differentiation and de-differentiation — a process where differentiated cells are reprogrammed back into undifferentiated pluripotent cells — and hence to tissue-engineering and regenerative-medicine applications. Writing in *Nature Materials*, Kevin Chalut and colleagues report the discovery of an unexpected biophysical phenotype of the nucleus of mouse ESCs in the metastable transition state: contrary to the behaviour of most known materials, the nucleus of these cells expands when stretched out¹.

To characterize the different physiological states of ESCs when they begin to differentiate, Chalut and collaborators used a green fluorescent protein (GFP) to report the expression of a specific pluripotency marker, *Rex1*. When a naïve ESC is cultured in a medium to maintain its pluripotency, *Rex1* expression is high, as visualized by the high expression of GFP. However, when soluble chemicals were replaced in the culture medium so as to induce differentiation, the compact colony of ESCs in the naïve state turned into a loose colony structure after 24 hours. At this transition stage, *Rex1* expression was about to change, while expression of a different pluripotency factor, *Nanog*, decreased dramatically. After 48 hours, the ESC colony started to form protrusions at the colony periphery, and *Rex1* expression shut down, which suggested that the cells were primed and committed to differentiation.

Chalut and co-authors probed the mechanical properties of ESCs in the naïve (N), transition (T), and primed (P) states with an atomic force microscope by indenting and compressing the cells' surface. They found that on compression the cytoplasm of all ESCs at the various states expanded in cross-section, as expected, but that the nucleus of ESC cells in the T state (T-ESCs) shrank, instead. The nucleus of T-ESCs thus exhibited a negative Poisson's ratio (which is defined as the negative of the normalized transverse-to-axial deformation ratio). When the authors placed T-ESCs under fluid flow in a microchannel that was narrow enough for the cells to expand in the direction of the flow, the cells' nucleus expanded in

both directions (Fig. 1). Interestingly, such an auxetic behaviour was exhibited only by nuclei in the T state; nuclei in the N or P states, or of lineage restricted cells, didn't behave as auxetic materials.

The driving mechanism for the auxetic behaviour of the nucleus of T-ESCs is, however, unclear. Chalut and co-authors observed that disruption of the actin cytoskeleton did not abolish auxeticity, which suggests that its origin resides in the cells' nucleus. In fact, decondensation of chromatin — the DNA and protein complex that forms the chromosomes — might be contributing to auxeticity, as a T-ESC's chromatin is less condensed than that of an N-ESC, and treating the N-ESC with a chemical to decondense chromatin made the N-ESC auxetic¹. It would thus be interesting to re-condense the nucleus of T-ESCs and see if this can abolish their auxetic behaviour. Also, it is not clear if the disappearance of auxeticity in the nucleus of P-ESCs is related to the re-condensation of the nucleus of P-ESCs. If this is the case, experiments could also be designed to decondense the nucleus of the P-ESCs so as to determine if auxetic behaviour is resumed. Moreover, the authors report that the nucleus of T-ESCs behaves as an auxetic material only for nuclear diameters of less than ~9 μm (this cut-off could be further examined by uniaxially stretching the cells with an optical stretcher²). It is thus possible that a decondensed chromatin inside a small nucleus contributes to auxeticity by exerting higher tension on the nuclear envelope. This interpretation is supported by observations of a smoother nuclear envelope in T-ESCs than in N-ESCs¹. Still, it is not clear how a tensed, smooth nuclear envelope is able to accommodate the need for extra surface area of the nuclear membrane when the nuclear volume increases under stretch.

Inside the nuclear envelope, the nuclear intermediate-filament proteins Lamin A/C and Lamin B provide viscous and elastic stiffness to the nucleus³. Lamin A enhances matrix-mediated differentiation⁴, and isolated nuclei require intact lamins to stiffen in response to forces⁵ directly applied to nesprin-1 (a nuclear-envelope protein that forms part of the protein complex LINC, which physically links nuclear lamins with actin microfilaments of the cytoskeleton). However, there are no detectable levels of Lamin A/C in ESCs, and no differences in the expression of the relevant genes (*Lmna* and *Lmnb1*) among N-, T-, and P-ESCs¹, suggesting that lamins may not contribute significantly to the auxeticity of the nucleus of T-ESCs. Although downregulation of the pluripotency factor *Nanog* accompanies the onset of nucleus auxeticity, *Nanog*-deficient cells can be reprogrammed from differentiated states back to induced pluripotent stem cells⁶ (iPSCs), thus casting doubts about *Nanog* having any major role in nuclear auxeticity. Still, it would be informative to determine whether (and when) *Nanog*-deficient iPSCs might exhibit an auxetic behaviour. It will also be interesting to determine if auxeticity can be used as an effective assay to access whether the nucleus of an iPSC is mechanically similar to that of an N-, T-, or P-ESC.

In addition to soluble chemical factors used to differentiate N-ESCs and to decondense chromatin, alterations in a cell's mechanical microenvironment might also induce nuclear auxeticity. In fact, it is well known that almost all cells respond to mechanical cues from the extracellular matrix⁷ (pluripotent ESCs do not stiffen with matrix rigidity⁸, yet their differentiation is preceded by shear-stress-induced spreading and stiffening⁹). It would thus be instructive to determine if the nucleus of an N-ESC behaves auxetically (presumably as it

goes through the T stage) when it differentiates as a result of external or matrix forces. Also, since an auxetic nucleus confers a different mechanical-deformation field to nuclear structures, auxeticity could induce significant changes in molecular transport across the nucleus envelope and in chromatin structure and transcription-factor conformation. It may well be that the nucleus of an inner-cell-mass cell in a developing embryo that exhibits auxetic behaviour responds differently than the normal nuclei of surrounding cells to local tension and compression (this could be quantified by an oil-droplet force transducer¹⁰), and that together with Wnt (and other soluble-factor) signalling pathways this results in physiological differentiation patterns and germ-layer organization.

Irrespective of the underlying mechanism, the finding of nuclear auxeticity for ESCs in the metastable T state represents a significant step toward in the understanding of the nucleus as a material. The work of Chalut and colleagues will facilitate future exploration of nuclear mechanics and mechanotransduction, chromatin structure and organization, transcription-factor activity, gene regulation and, ultimately, cellular functions.

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Figure 1.

The nucleus of ESCs in the transition state expands when stretched. When a T-ESC moves under fluid flow within a microchannel (indicated by the green lines) that is narrower than the cell's diameter, compressive forces cause the cell's cytoplasm (blue), — and thus the viscoelastic cytoskeleton (symbolically depicted by red coils) — to shrink perpendicularly to the flow and to stretch in the flow's direction (arrow) so as to preserve the cell's volume. However, the cell's nucleus (orange) expands in all directions, thereby increasing its volume. Hence, the nucleus exhibits auxetic behaviour (its Poisson's ratio is thus negative).