In eukaryotic cells, nuclear pore complexes (NPCs) fuse the inner and outer nuclear membranes and mediate nucleocytoplasmic exchange. They are made of 30 different nucleoporins and form a cylindrical architecture around an aqueous central channel. This architecture is highly dynamic in space and time. Variations in NPC diameter have been reported, but the physiological circumstances and the molecular details remain unknown. Here, we combined cryo–electron tomography with integrative structural modeling to capture a molecular movie of the respective large-scale conformational changes in cellulo. Although NPCs of exponentially growing cells adopted a dilated conformation, they reversibly constricted upon cellular energy depletion or conditions of hypertonic osmotic stress. Our data point to a model where the nuclear envelope membrane tension is linked to the conformation of the NPC.

Nuclear pore complexes (NPCs) bridge the nuclear envelope (NE) and facilitate nucleocytoplasmic transport. Across the eukaryotic kingdom, ~90 different genes encode for NPC components, termed nucleoporins (Nups). Although specialized Nups have been identified in many species, extensive biochemical and structural studies have led to the consensus that the core scaffold inventory is conserved. It consists of several Nup subcomplexes that come together in multiple copies to form an assembly of eight asymmetric units, called spokes, that are arranged in a rotationally symmetric fashion (7). The Y-complex (also called the Nup107 complex) is the major component of the outer rings (the nuclear ring (NR) and the cytoplasmic ring (CR)), which are placed distally into the nuclear and cytoplasmic compartments. The inner ring complex scaffolds the inner ring ([IR] also called the spoke ring) that resides at the fusion plane of the nuclear membranes. It consists of the scaffold Nups 35, 155, 188, and 192 as well as the Nup1 complex. The IR forms a central channel lined with phenylalanine-glycine (FG) repeats containing Nups that interact with cargo complexes. The Nup159 complex (also the Y-complex) asymmetrically associates with the Y-complex of the CR and mediates mRNA export. Despite these common features of quaternary structure, in situ structural biology studies have revealed that the higher-order assembly is variable across the eukaryotic kingdom (1, 2).

NPC architecture is conformationally highly dynamic, and variations in NPC diameter have been observed in various species and using different methods (3–7). Dilated states have been observed in intact human cells (3, 8, 9), contrasting with the constricted state in semi-purified NPCs (10–12). It has been shown that dilation is part of the NPC assembly process (13, 14). However, it remains controversial whether NPC dilation and constriction play a role during active nuclear transport (15) and whether the dilation is required to open up peripheral channels for the import of inner nuclear membrane (INM) proteins (16–18). It has been argued that the constricted state may be a result of purification (4, 19). It is difficult to conceive that such large-scale conformational changes can occur on similar time scales as individual transport events (19, 20), which would be the essence of a physical gate. Nevertheless, several cues that potentially could affect NPC diameter have been suggested, such as exposure to mechanical stress, mutated forms of importin β, varying Ca2+ concentrations, or exposure to hexane/diol (7, 21–26). The biological relevance of these cues remains elusive because the analysis of NPC architecture under physiological conditions is experimentally very challenging. Previous studies did not explore NPC dilation and constriction and its functional cause and consequences within intact cellular environments, nor did they structurally analyze the conformational changes of nuclear pores in high molecular detail.

Here, we demonstrate that Schizosaccharomyces pombe NPCs (SpNPCs) constrict in living cells under conditions of energy depletion (ED) or hyperosmotic shock (OS), which is concomitant with a reduction of NE membrane tension. Using in cellulo cryo-electron microscopy (cryo-EM) and integrative structural modeling, we captured a molecular movie of NPC constriction. Our dynamic structural model suggests large-scale conformational changes that occur by movements of the spokes with respect to each other but largely preserve the arrangement of individual subcomplexes. Previous structural models obtained from isolated NEs (10–12, 27–29) thereby represent the most constricted NPC state.

In cellulo cryo-EM map of the SpNPC

To study NPC architecture and function in cellulo at the best possible resolution and structural preservation, we explored various genetically tractable model organisms for their compatibility with cryo–focused ion beam (cryo-FIB) specimen thinning, cryo–electron tomography (cryo-ET), and subtomogram averaging (STA). *Saccharomyces cerevisiae* cells were compatible with high-throughput generation of cryo-lamellae and acquisition of tomograms. STA of their NPCs resulted in moderately resolved structures (4). By contrast, a larger set of cryo-tomograms from *Chlamydomonas reinhardtii* cells did not yield any meaningful averages, possibly because their NPCs display a very large structural variability. We therefore chose to work with *S. pombe* cells that are small enough for thorough vitrification; offer a superior geometry for FIB milling compared with *C. thermophilum*, with the advantage of covering multiple cells; and, compared with *S. cerevisiae*, have a higher number of NEs and NPCs per individual cryo-lamella and tomogram, leading to increased data throughput (fig. S1).

To obtain a high-quality cryo-EM map of SpNPCs, we prepared cryo-FIB–milled lamellae of exponentially growing *S. pombe* cells and acquired 178 tomograms from which we extracted 726 NPCs. Subsequent STA resulted in an in cellulo NPC average of very high quality in both visible features and resolution (Fig. 1 and figs. S2 and S3). Systematic fitting of the *S. pombe* IR asymmetric unit model, built based on the *S. cerevisiae* NPC, resulted in precisely one highly significant fit, as expected (figs. S4A and S5A and Materials and methods). The subsequent refinement with integrative modeling led to a structural model that explains most of the observed EM density in the IR (Fig. 1B, fig. S2B, and movie S1). The IR architecture appears reminiscent to NPC structures of other eukaryotes (fig. S6), further corroborating its evolutionary conservation (23–30) (see table S1 for nomenclature of Nups across different species). Systematic fitting revealed that the NR of the SpNPC is composed of two concentric Y-complex rings (Fig. 1A, 1B, and movie S1).