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Macromolecular assemblages — from molecules to functional modules

Editorial overview

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Over the past decade, awareness has grown that cells are more than collections of individual macromolecules [1]. Single proteins can catalyze biochemical reactions, but higher cellular functions depend on carefully orchestrated protein interaction networks. As in a factory, these cellular networks are organized in a hierarchical manner and comprise arrays of functional modules. Some of these modules are tightly integrated, and withstand the isolation and purification procedures that are traditionally used in biochemistry. Other supramolecular functional modules exist only transiently; often they are promiscuous in their subunit composition and undergo remodeling in response to specific signals. Here, the challenge for the structural biologist is to isolate or reconstitute such modules in a physiologically relevant configuration and under conditions amenable to structural studies [2].

Expression of the genetic information in eukaryotes relies on the sequential action of large and dynamic macromolecular assemblies. First, regions of condensed chromatin must be rendered accessible to the transcription machinery. The transcription machinery then locates to activated genes and synthesizes mRNA, which is subsequently processed and exported into the cytoplasm. The mature mRNA is translated into a polypeptide chain by the ribosome. As the nascent chain emerges from the ribosome, chaperones assist in adopting the correct three-dimensional fold. Alternatively, the signal recognition particle can recruit active ribosomes with nascent polypeptides to the endoplasmic reticulum. The protein is inserted into the endoplasmic reticulum, where it is modified before its further distribution within the cell. The structural organization and dynamics of several macromolecular assemblies involved in gene expression are summarized in four reviews in this section of *Current Opinion in Structural Biology*.

Luger and Hansen review the dynamics of chromatin fibers and the nucleosome, its underlying building block. The authors review recent biochemical and biophysical data on the assembly and disassembly of nucleosomes and higher order chromatin structures, and show that nucleosomal assemblages are inherently dynamic at all levels of structural organization. Of particular interest is the protein-mediated assembly and disassembly of chromatin and nucleosome structures, because they play important roles in the regulation of gene expression.

Armache, Kettenberger and Cramer summarize our current understanding of the dynamic transcription machinery during the phase of mRNA elongation. In particular, they compare new three-dimensional structures of RNA polymerase II bound to template DNA and product RNA, and describe

current models of the mechanism of binding and incorporation of nucleoside triphosphate substrates into the growing RNA chain. They also show how the 'tunable' active site of the polymerase, together with extrinsic factors, allows modulation of enzyme activity during gene transcription.

Ban and co-workers report on the mechanism of the trigger factor, a ribosome-associated chaperone that helps the emerging nascent polypeptide chain to adopt its correct three-dimensional fold. Recent structures of the trigger factor in free and ribosome-bound forms show that it forms an additional reaction compartment at the exit of the ribosomal tunnel. This compartment sequesters newly synthesized proteins that emerge from the tunnel and thereby protects them from unproductive interactions in the crowded environment of the cell. Interpretation of the new structures in conjunction with biochemical data leads to the conclusion that the specific shape of the trigger factor may allow cooperation with additional factors involved in cellular protein folding.

Egea, Stroud and Walter report on the structure of signal recognition particles and their implications for understanding the targeting of proteins to membranes. The authors review structures of parts of the signal recognition particle and discuss the conservation of the particle over the three kingdoms of life. Based on EM data, they also put the signal recognition particle into a cellular framework and discuss the mechanistic basis of its function on the ribosome. Finally, the authors review our current understanding of how GTPase activity regulates membrane targeting of complexes of the elongating ribosome with the signal recognition particle.

One review, by Schwartz, summarizes recent developments in dissecting the molecular architecture of the nuclear pore complex (NPC) and puts them into perspective. In spite of intensive efforts in several laboratories, our understanding of the structure and molecular mechanism of the NPC gate, which controls all nucleocytoplasmic transport, is still substantially incomplete; given the extraordinary size of NPCs, their location in the membranous environment of the nuclear envelope and their dynamics, this is not surprising. There is no way a single method could provide us with a comprehensive picture. Schwartz highlights three aspects of nuclear pore structure. Cryo-electron tomography has offered a means of visualizing NPCs in their functional environment; this has greatly reduced the risk of artifacts and has yielded a fairly detailed overall picture. Moreover, it gave a first glimpse of the structural changes that are associated with the translocation of cargo. Advanced light microscopy studies have demonstrated that only a subset of the molecular components — the nucleoporins — are stably integrated into the NPC structure, whereas several others shuttle on and off in a timeframe of seconds. X-ray crystallography has provided us with the first high-resolution structures of the molecular components. Surprisingly, a limited repertoire of domain structures, most notably α-helical repeats, β-propellers and coiled coils, appear to make up the nucleoporins, reminiscent of the structural organization of the recently determined structure of clathrin-coated vesicles [3].

Two reviews report advances in understanding virus structure. Steven *et al.* discuss virus assembly and maturation, that is to say, the intriguing sequence of events that transforms a provirion into an infectious particle. With a focus on capsid formation, they describe the interplay between capsid proteins and scaffolding proteins, the role of the DNA translocating machinery (the 'connector'), as well as the role of viral proteases and other accessory proteins that trigger and orchestrate the structural changes. In recent years, high-resolution structures of several representative examples of these proteins have been obtained and time-resolved cryo-EM studies have allowed their integration into a common mechanistic framework. Virus assembly and maturation involve large-scale structural changes ranging from rigid-body movements to local refolding and redeployment of motifs. Maturation movies provide us with vivid pictures of the structural transformations that yield infectious virions.

Adenovirus has served as a paradigm for understanding the structure of complex viruses — its proteome comprises approximately 45 proteins — and their interactions with host cells. Moreover, it is of current interest as a medium for gene transfer. Cusack reviews recent structural work on adenovirus capsid proteins and their implications for the infection process. Hybrid X-ray crystallography and cryo-EM studies have allowed the synthesis of a pseudo-atomic model of the adenovirus capsid, and surprising structural similarities have been found between adenovirus and several quite unrelated viruses. Most unexpected and intriguing, however, is the discovery of structural similarities between the adenovirus cell attachment proteins — the penton protein and the penton base — and the primary host cell receptors.

From the collection of reviews in this section of *Current Opinion in Structural Biology*, it is obvious that hybrid approaches combining high-resolution structures of molecular components with low-resolution structures of whole assemblages become increasingly important. Cryo-EM will play a key role in furnishing medium-resolution structures of protein assemblies; it has the advantage that requirements in terms of sample quantity and purity are very modest. Structures with a resolution of approximately 10 Å can now be obtained routinely and the prospects for further improvements are good [4]. Once slow and a specialized craft, high-throughput tactics have been developed and will greatly accelerate the pace at which structures of otherwise elusive large functional

modules become available. Quantitative computational methods are under active development, allowing the interpretation of such medium-resolution density maps in terms of the accurate placement of high-resolution structures of subunits or subcomplexes, yielding pseudo-atomic maps of large assemblages [5].

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