Dissecting the Function of Biological Machines Using Fleezers

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Optical tweezer has been a classic method widely applied in the biophysical study for decades. Optical forces originate from the momentum exchange when light interacts with an object[1], and the forces include the gradient force, which is produced by the polarization of an object in a non-uniform electronic field, and the scattering force, which is produced by the absorption and the scattering of light. Stable optical trappings are achieved when the gradient force is balanced with the scattering force[1]. The detection of gradient and scattering forces on micron-sized particles was first reported by Professor Arthur Ashkin in 1970[2] and was applied to the cooling and trapping of neutral atoms by Steven Chu in 1986[3]. The standard optical tweezers yield rich information on the mechanical behaviors of the molecules by real-time recordings of force and extension[1]. However, there are still some limitations. For example, only with the standard optical tweezers, it's not easy to measure the position and the orientation of single biomolecules, as well as their conformational fluctuations. Now this limitation can be overcome by the combination with fluorescence imaging. The hybrid method enables measurements of mechanical signals and forces simultaneously with fluorescence imaging of the same system[1]. The property of real-time measurement and imaging is ideal for single-molecule tracking, and thus for studying the activity of molecular motors and the dynamics of biochemical processes.

In a 2019 paper from Molecular Cell, Desai et. al used optical tweezers with single-molecule fluorescence detection to investigate how ribosomes unwind mRNA secondary structures to overcome the structural obstacles by interacting with the translocation factor EF-G[4]. In this study, forces were first applied to unwind the hairpin of mRNA. Combined with the measurement of fluorescently labeled EF-G, they first proved that hairpin unwinding occurs always after EF-G arrival[4]; similarly, the release of EF-G occurs always after the unwinding event[4]. This observation ends the debate about the orders of EF-G binding and mRNA hairpin unwinding. In the next step, they modulated the strength of the hairpin by applying force to destabilize the junction[4]. so the higher the force, the weaker the hairpin. And they found that the rate of translation decreases 2-fold as the strength of the downstream hairpin increases[4]. To further study how ribosomes respond to the hairpin strength, they analyzed the single ribosome trajectory. The results showed that fitting of the distribution of the dwell time, the time between EF-G binding and hairpin unwinding, and the unwinding time, the time between hairpin unwinding and hairpin release, all required a minimum of two exponentials[4]. That indicates that the kinetic mechanism of the ribosome elongation cycle is not a simple linear scheme but that it bifurcates into two alternative pathways with two different translation rates[4]. And the stability of the hairpin junction determines the fraction of time that the ribosome chooses which pathway to go[4]. Therefore, the whole process of how ribosome changes its conformation with the interaction of EF-G to

unwind mRNA secondary structure is clear: First, there is a rapid and irreversible "hairpin sensor" step during which the ribosome chose between the "fast" and "slow" pathways based on the strength of the downstream hairpin[4]. Second, an intermediate kinetic step occurs in both pathways which are force-insensitive and determine the rates of translation[4]. In the next step, in the presence of EF-G, the ribosome opens the hairpin[4]. After the unwinding event, the two pathways converge, EF-G is released, and the ribosome is reset for another round of elongation[4].

The successful functioning of cells depends on proper protein folding. Inappropriate folding or misfolding can lead to the formation of protein aggregate which is often correlated with some diseases such as Alzheimer's[5]. In the cell, there are some safeguards that can protect the cell from the protein aggregate[5]. Disaggregase is a type of protein that has the ability to dissolute and clear the already formed protein aggregates. ClpB disaggregase, a member of the Hsp100 disaggregases family, is proposed to catalyze the disaggregation reaction by translocating polypeptide loops through their central hole[6]. However, the unambiguous evidence of necessary molecular motor activity is still missing[6]. A 2020 paper from Nature completes the translocation-involved disaggregation model by integrating optical tweezers with fluorescent-particle tracking to observe the translocation process of looped polypeptides during the protein disaggregation[6]. In this study, the standard optical tweezer was first applied to unfold the protein and then was reduced to prevent its spontaneous refolding[6]. The mechanical signals showed ClpB produced processive runs during the translocation[6]. And then by additionally labeling the ClpB protein by fluorescence, they measured the length change of the left and right arms of the polypeptides loop during the translocation independently[6]. Analysis of the mechanical and fluorescence channels indicates that ClpB can translocate both arms simultaneously and switch to a single-arm translation pattern when encountering obstacles[6]. What's more, a segment of about 270 aa remaining on the trans side of ClpB was observed in the experiment, suggesting that polypeptide folded and was subsequently blocked at the trans side of ClpB pore[6]. The refolding only happens after a certain region of the polypeptide is released to the trans side. Putting it all together, this study defines the loop extrusion mechanism of Hsp100 disaggregation and provides evidence of the refolding of substrates.

The selected examples show the great potential of fluorescence-combined optical tweezers (or "fleezer") in the study of DNA/RNA-protein interactions. The ability of fleezer to "see" fluorescently labelled molecules allows the determination of their location in the systems as a function of time and applied force, and thus can be used for characterizing the molecular trajectories over time and revealing the mechanism of how they function.

Reference

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