Novel insights into filament-forming enzymes

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Enzyme filaments are defined as reversible, functional, linear self-assemblies of a single type of enzyme. Filamentation has recently emerged as a new mode of enzymatic regulation. In this Comment, we briefly introduce the diversity and functional consequences of enzyme filamentation.

Filamentation was noted nearly 50 years ago in studies of enzymes purified from natural sources, and filaments were characterized using various biophysical techniques, including electron microscopy[1]. As X-ray crystallography emerged as the method of choice for structural studies of proteins, enzymes were chosen for study based on the ability to produce high-quality crystals, which precluded most filament-forming enzymes and/or filament-forming conditions. However, in the past 10 years, several large-scale screens have identified more than 60 different enzymes, from diverse organisms (bacteria, yeast, flies and mammals) and metabolic pathways, that form large self-assemblies in cells that are visible using fluorescence microscopy[2,3]. These cellular self-assemblies appear as rods, rings, fibres or foci. It is important to note that the screens have been designed to select only reversible self-assemblies, to differentiate them from aggregates of unfolded proteins. The screens also show that enzyme self-assembly is often associated with stress conditions such as nutrient starvation, yet can also be seen in some cases in normal physiological conditions. In vitro studies are now beginning to reveal the diversity of the mechanisms of enzyme regulation through filamentation.

Assemblies and filament structures

To date, more than 20 different enzymes have been shown to form reversible, filamentous structures in vitro. Many of the same enzymes that form self-assemblies in cells also form filaments in vitro, under the same or related conditions. Many self-assemblies seen in cells are thought to be composed of filamentous forms of the enzymes, either in organized, close-packed bundles or in looser, more gel-like networks. Yet, few studies have addressed the detailed structure of the majority of the more than 60 enzymes known to form cellular self-assemblies in vivo.

In vitro structures of several filamentous enzymes have been determined at resolutions that allow the generation of detailed molecular models[4–6]. Enzyme filaments are heterogeneous in nature and tend to crystallize poorly, hence most structures rely on electron microscopy techniques for structure determination. Many of the protomers of the enzyme filaments are themselves well-defined oligomers (dimers, tetramers, hexamers), which form the repeating units of the filament. In general, enzyme filaments can be helical, linear or composed of stacks of rings. Some helical filaments are even seen to form paired or triplet helices. The helical filaments can be either right-handed or left-handed, and in some cases they have pitches that result in tight junctions between coils of the helix that create cylinders, sometimes with large central lumens.

The effects of filamentation

The effect of filamentation on enzyme activity has been studied in detail in only a handful of cases. Some enzymes are inactivated, whereas others are activated in the filamentous state. Some enzymes form more than one type of filament, with opposite effects on their enzymatic activity. Filamentation may therefore serve as a way to stabilize and reinforce the active or inactive state of the enzyme, although the advantage of using filamentation over other forms of allostery is not fully understood. One advantage of regulating enzyme activity by filamentation may be more rapid, cooperative activation or inhibition in response to the binding of substrate, product, allosteric effectors, ligands, other proteins or nucleic acids, and/or in response to post-translational modification. Alternatively, an equilibrium between filaments of inactive and active enzyme could control or buffer the levels of enzymatic activity and/or control the maximum turnover rate of enzymes. In at least three cases, filamentation has been found to control the substrate specificity of an enzyme either through steric occlusion of larger substrates to an interior active site, or by creating substrate binding sites of different sizes, which favour larger or smaller substrates depending on the helical periodicity of the filament[7]. Another filament induces a secondary enzymatic activity by drawing in enzymes bound to a secondary substrate, thereby activating the filament-bound enzyme towards these substrates[6].

Enzyme filamentation could have roles beyond controlling enzyme activity. In at least one case, the

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filamentous form takes on an entirely new function. The enzyme 2-Cys peroxiredoxin protects cells against oxidative stress at low-stress levels, but at high-stress levels it becomes a protein chaperone by forming a hollow filament that binds unfolded proteins[8]. During starvation or oxidative stress, the filamentation of other enzymes may serve to protect them against degradation, thereby allowing rapid restoration of enzymatic activity upon stress release. Finally, filaments can function in signalling pathways by providing a platform for the binding of signalling enzymes, as in the case of filament-activated kinases that function in innate immunity responses[9].

The kinetic advantages of filaments

Few studies to date have investigated the advantages of filament formation for enzyme regulation, especially in comparison with other regulatory mechanisms. One exception has been the studies of the protein SgraIR of the bacterium *Streptomyces griseus*. SgraIR (also known as SgrAI) is a type II restriction endonuclease that is allosterically activated by binding to a primary recognition sequence of DNA. This results in enzyme filamentation, additional enzyme activation and expansion of its DNA-binding specificity to include a secondary set of DNA sequences. Global kinetic modelling of the full reaction pathway of SgraIR, including filament formation, has provided a computational model of the full enzymatic reaction, complete with microscopic rate constants for each step. Use of this kinetic model has enabled the simulation of activity in vivo and its comparison with that of non-filament-forming enzymes[10]. Interestingly, enzyme regulation through filamentation provides a unique advantage in the rapid activation of SgraIR. The advantage is derived from the activation of SgraIR by joining to a growing filament at either end, rather than by using a single interface as in the case of the non-filament form. Filament formation is also advantageous to the cleavage of the secondary recognition sequence of SgraIR, as no competition in binding to the filament occurs between SgraIR molecules that are bound to the two types of recognition site (primary and secondary, which have different affinities for the filament) owing to the continuously growing filament.

Importantly, the association of SgraIR with the filament ends is governed by a slow second-order rate constant, which can be overcome by high local concentrations of the DNA substrate, for example, when multiple recognition sites occur on the same contiguous DNA (such as on invading phage DNA)[10]. This slow association rate constant limits incorporation of SgraIR enzymes bound to recognition sites on separate DNA molecules and favours filament formation by enzymes bound to recognition sites on the same contiguous DNA. Such sequestration of enzyme activity, namely cleavage of only a particular DNA molecule, is crucial for the biological role of SgraIR in protecting *S. griseus* from invading DNA without damaging its own DNA. Mutations that disrupt SgraIR filamentation, even moderately, completely abrogate measurable protection against phage infection in vivo[10]. These results show how the rapid activation of SgraIR is crucial to its function and provide an explanation for why filament formation evolved in this enzyme.

Future perspective

Filamentation appears to be an important, evolutionarily conserved enzyme regulation mechanism, which is only now beginning to be understood. More studies of the role of filamentation in the regulation of enzyme activity are required to understand the breadth of regulatory mechanisms made possible by filamentation and to uncover the mechanistic advantages of this particular phenomenon. Connecting studies of enzyme filamentation with those of the formation of large self-assemblies in cells is also an important area of investigation, which will determine how and why enzyme filaments organize into such subcellular compartments and what special properties these compartments may have in cell function.

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Competing interests
The authors declare no competing interests.