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Method Article

Induction of protein aggregation in zebrafish embryos as a method for the screening of new drugs or mutations against proteinopathies

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A B S T R A C T

The sustained increase in the prevalence of protein aggregation related diseases requires the development of feasible methods for the design of therapeutic alternatives. The procedure traditionally used for the search of drugs or therapeutic mutations includes *in vitro* experiments, designed to prevent the aggregation of model proteins, which are then complemented with cellular toxicity studies *in vivo*, slowing down the finding of solutions. To address this, we have developed a protocol that facilitates the search of molecules and anti-aggregation mutations since it allows to evaluate their therapeutic capabilities directly in *in vivo* experiments with the use of zebrafish early embryos. Avoiding the necessity of performing *in vitro* and *in vivo* procedures separately. Giving a more realistic method for the results interpretation.

Zebrafish embryos were induced to produce intracellular aggregates of proteins by simple microinjections of known quantities of an aggregation prone protein previously labeled. The toxicity was evaluated by the survival of the embryos, while the formation of aggregates was quantified by fluorescence microscopy. The size distribution of the protein aggregates was revealed by means of ultracentrifuge sedimentation analysis.

For the development of the present method, the human γ-tubulin protein was used as model protein, which generated intracellular aggregates in more than 60% of the injected embryos. To evaluate the method, a mutation was performed that altered the state of intracellular aggregation of γ-tubulin, obtaining a significant decrease in the amount and size of the intracellular aggregates. The present method can be used for any suitable intracellular aggregation protein model.

The current method present important advantages such as:

Easy induction of intracellular aggregates.

Simple detection of intracellular protein aggregates through fluorescence microscopy and subcellular fractionation.

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Overall view of the effect of drugs or mutations by combining the toxicity, the development behavior and the size distribution of intracellular protein aggregates.

ARTICLE INFO
Method name: Induction of protein aggregates in zebrafish early embryos by direct microinjection of denatured protein
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Specifications Table

Subject area	Biochemistry, Genetics and Molecular Biology
More specific subject area	Protein aggregation
Method name	Induction of protein aggregates in zebrafish early embryos by direct microinjection of denatured protein.
Name and reference of original method	The in vivo novel method developed here uses microinjection of proteins into zebrafish embryos to screen drugs or mutations that interfere their aggregation. The original methods used *in vitro* protocols to follow protein aggregation through spectroscopy by the binding of specific probes. Paslawski W., Lorenzen N., Otzen D.E. (2016) Formation and Characterization of α-Synuclein Oligomers. In: Eliezer D. (eds) Protein Amyloid Aggregation. Methods in Molecular Biology, vol 1345. Humana Press, New York, NY.
Resource availability	

Method details

Background

The method involves the induction of zebrafish zygote-early embryo to form large intracellular aggregates induced by exogenous proteins injected into the cytoplasm by direct microinjection. The aggregation process is likely to trigger the activation of general pathways of protein misfolded response [1], including the interaction with molecular chaperones and post translational modifications. Regardless of the identity of the injected protein, or the particular way of aggregation, the intracellular aggregates can be easily detected by fluorescent microscopy. Furthermore, the toxicity can be quantified counting the survival embryos, allowing dose-effect analysis. The size distribution of the aggregates can be analyzed by cytoplasm fractionation through sedimentation in glycerol density gradient using ultracentrifugation.

Protein preparation

Protein purification

An aggregation-prone protein should be used as an aggregation model. The purification protocol will depend on the desired protein. Recombinant human γ-tubulin protein was used for the development of the present protocol. The protein was purified to homogeneity with purity higher than 98% (for recombinant γ-tubulin purification and aggregation refer to Pouchucq et al. [2]). Considering that γ-tubulin will naturally tend to aggregate, it is necessary to keep it denatured in solution by using denaturant buffer (8 M urea, 10 mM $Na_2S_2O_5$, 5 mM DDT, 20 mM Tris, pH 8).