Nucleic Acids Chemistry beyond the Watson-Crick Double Helix (37): The formation of RNA foci in repeat expansion disorders is promoted in molecular crowding conditions

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The expansion of d(GGGGCC) repeats in the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). [1] The transcribed r(GGGGCC) repeats form RNA foci by phase transition both *in vitro* and in cells, which are composed of intermolecular non-canonical G-quadruplex and hairpin structures.[2] We have demonstrated that physical properties of nucleic acids in the molecular crowding condition had drastic differences relative to dilute solution.[3] It is noteworthy that the formation of RNA foci should be associated with cellular environments.

In this study, we investigated the effects of surrounding conditions on the gelation of $r(GGGGCC)_8$ characterized by turbidity and particle size. Three stages were observed in the formation of RNA foci in $r(GGGGCC)_8$, including RNA accumulation, gelation and sedimentation. The presence of toxic peptide $(GR)_4$ which is encoded by r(GGGGCC) repeats can promote the gelation. More importantly, the crowding conditions induced by cosolutes, such as glycerol, polyethylene glycol, dextran and ficoll, were demonstrated to significantly accelerate the gelation process. In the tested cosolute of the largest effect, the sedimentation occurred after 1.55 h, while in dilute case it occurred after 8.7 h. Interestingly, the gelation rate increased with the decrease of the dielectric constant, indicating the importance of electrostatic interactions in the formation of RNA foci. The observed effects of the encoded peptide and crowding conditions on gelation would give us new information about the mechanism involved in neurodegeneration in repeat-associated diseases.

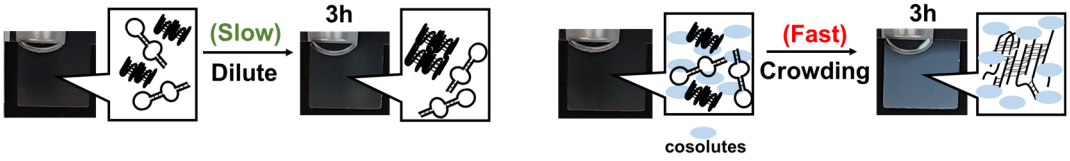

Figure. The photos and schematic demonstrations of the gelation of RNA repeats after 3 h of reaction in dilute (left) and crowding (right) conditions.

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