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СТРУКТУРНО-ФУНКЦИОНАЛЬНЫЙ АНАЛИЗ БИОПОЛИМЕРОВ И ИХ КОМПЛЕКСОВ

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РАЗДЕЛЕНИЕ ФАЗ В ПРЕПАРАТЕ ОЧИЩЕННОГО БЕЛКА ЧЕЛОВЕКА LSM4[1]

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Жидкофазное разделение белков происходит в ряде биологических процессов, таких как регуляция транскрипции, процессинг и созревание РНК. Sm-подобный белок 4 (LSM4) участвует во множестве процессов, включая сплайсинг пре-мРНК и сборку Р-телец. Перед проведением исследований по участию LSM4 в разделении двух жидких фаз в ходе процессинга или созревания РНК необходимо сначала обнаружить эту способность белка LSM4 в системе *in vitro*. Плазмиду, экспрессирующую белок mCherry-LSM4, получали на основе вектора pET30a и использовали для выделения белка mCherry-LSM4 из прокариотических клеток (*Escherichia coli* штамм BL21). Белок mCherry-LSM4 очищали с использованием аффинной хроматографии на Ni-NTA-агарозе, а затем жидкостной хроматографии быстрого разрешения (FPLC). Широкопольную флуоресцентную микроскопию Delta-Vision использовали для наблюдения за динамическим разделением фаз жидкость–жидкость в препаратах белка LSM4 *in vitro*. В ходе анализа структуры белка LSM4 с помощью базы данных Predictor of Natural Disordered Regions установлено, что его С-конец содержит домен низкой сложности. Из *E. coli* получен очищенный препарат полноразмерного белка LSM4 человека. Показано, что LSM4 человека обеспечивает зависимое от концентрации разделение фаз жидкость–жидкость *in vitro* в буфере с краудинг-агентами. Соли в высокой концентрации и 1,6-гександиол блокируют LSM4-индуцированное разделение двух жидких фаз. Кроме того, *in vitro* наблюдается слияние капель белка LSM4. Полученные результаты свидетельствуют о том, что полноразмерный белок LSM4 человека обладает способностью образовывать жидкие включения и вызывать разделение фаз жидкость–жидкость *in vitro*.

Ключевые слова: разделение фаз жидкость–жидкость, Sm-подобный белок 4, жидкостная экспресс-хроматография белков, очистка белков, биофизический процесс

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Phase Separation of Purified Human LSM4 Protein

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Liquid–liquid phase separation of proteins occur in a number of biological processes, such as regulation of transcription, processing, and RNA maturation. Sm-like protein 4 (LSM4) is involved in multiple processes, including pre-mRNA splicing and P-bodies assembly. Before investigating the involvement of LSM4 in the separation of the two liquid phases during RNA processing or maturation, the separation of the liquid phases in an *in vitro* preparation of LSM4 protein should be first be detected. The mCherry-LSM4 plasmid was derived from pET30a and used to isolate mCherry-LSM4 protein from prokaryotic cells (*Escherichia coli* strain BL21). The mCherry-LSM4 protein was purified using Ni-NTA resin. The protein was further purified by fast protein liquid chromatography. Delta-Vision wide-field fluorescence microscopy was used to observe the dynamic liquid–liquid phase separation of the LSM4 protein *in vitro*. Analysis of the LSM4 protein structure using the Predictor of Natural Disordered Regions database revealed that its C-terminus contains a low complexity domain. A purified preparation of full-length human LSM4 protein was obtained from *E. coli*. Human LSM4 was shown to provide concentration-dependent separation of liquid–liquid phases *in vitro* in buffer with crowding reagents. Salts in high concentration and 1,6-hexanediol block the LSM4-induced separation of the two liquid phases. In addition, *in vitro* fusion of LSM4 protein droplets is observed. These results indicate that the full-length human LSM4 protein has the ability to form liquid inclusions and induce liquid–liquid phase separation *in vitro.*

Keywords: liquid–liquid phase separation, Sm-like protein 4, fast protein liquid chromatography, protein purification, biophysical process