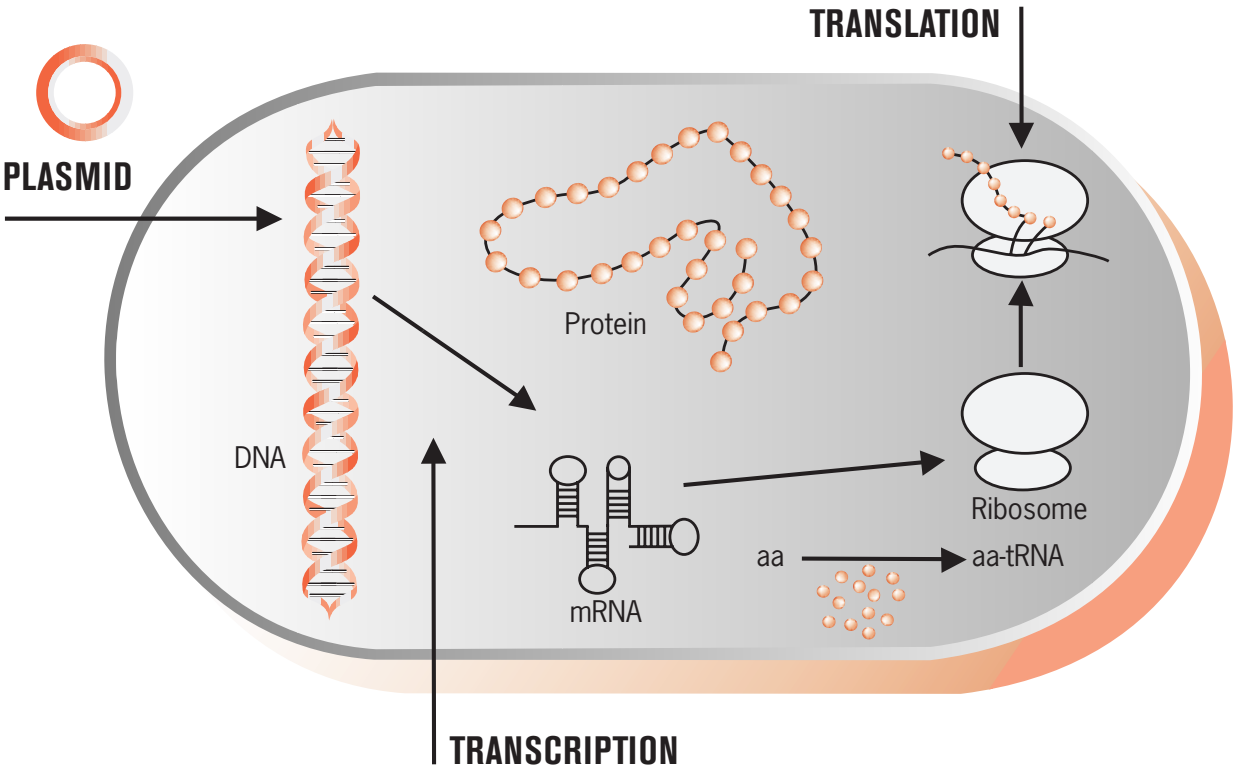
Silantes
Stable Isotope Labeled Biomolecules

IN-VITRO SYNTHESIS

RiNA GmbH
NETZWERK RNA-Technologien

IN-VITRO-SYNTHESIS OF STABLE ISOTOPE LABELED PROTEINS



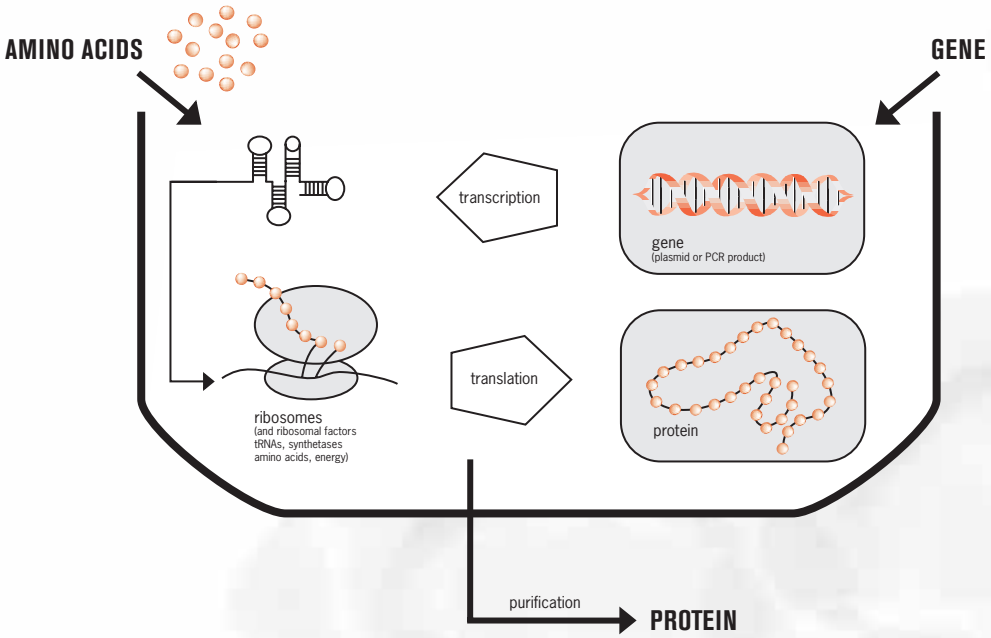
IN-VITRO-SYNTHESIS OF PROTEINS

BACKGROUND

Silantes in collaboration with RiNA offers in-vitro synthesis of isotopically labeled proteins as a service to customers. RiNA is an expert in the in-vitro synthesis of proteins and Silantes specializes in the production of stable isotope labeled biomolecules.

TECHNOLOGY

The in-vitro synthesis of proteins is performed in a cell free system that contains all necessary components for the transcription and translation of proteins. These components are purified from a bacterial lysate. The gene carrying the information of the expressed protein and the amino acids required for synthesis of the labelled protein are added to the lysate as indicated in the Fig. below. The labelled protein is purified from the lysate.



ADVANTAGES

In-vitro synthesis of proteins is an alternative to in vivo expression when the protein tends to:

- be toxic
- precipitate in cells as inclusion bodies
- be sensitive to proteolytic digestion in cells

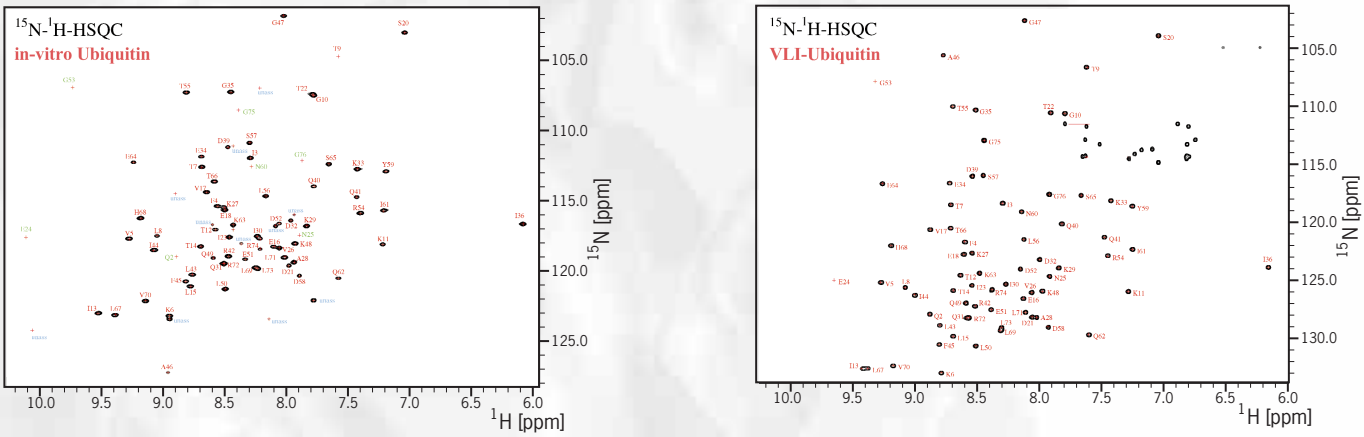
The in-vitro synthesis offers new possibilities for structural Bio-NMR:

- fully stable isotope labeled proteins can be synthesized
- a single amino acid species can be labeled in an otherwise unlabeled protein
- the protein can be partially labeled (free choice of amino acid species)
- the protein can be synthesized in mg-quantities sufficient for NMR analysis

PARTIALLY LABELED UBIQUITIN

Human ubiquitin containing a strep-tag II at the C-terminus was synthesized in- vitro by a cell free expression system:

- Ubiquitin was synthesized from a mixture of isotopically ($^{13}C/^{15}N$, purity > 98%) labeled amino acids.
- Trp, Asn and Gln were unlabeled.
- The purification was performed in two steps using streptavidin affinity chromatography and gel filtration.
- The amount of obtained protein was 2.5 mg.
- The yield was 60%.



Comparison of HSQC spectra of human ubiquitin synthesized in-vitro (left) and obtained commercially as human ubiquitin standard (right):

This study is the result of a collaboration of:

1. Max-Planck-Institut für Biochemie, Martinsried, Germany
2. Institut für Biochemie, Freie Universität Berlin, Berlin, Germany
3. Johann Wolfgang Goethe University, Large Scale NMR Facility, Aventis/Goethe-University Cooperation, Frankfurt, Germany
4. RiNA GmbH, Berlin, Germany
5. Silantes GmbH, Munich, Germany

CONCLUSION

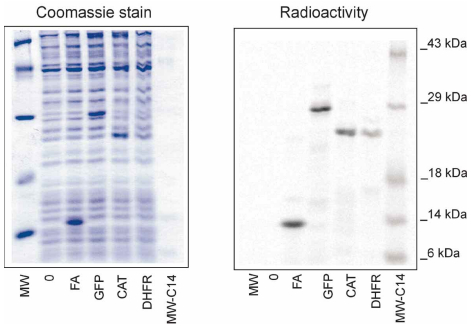
Our study demonstrates that it is possible to synthesize isotopically labeled proteins in-vitro in quantities sufficient for NMR-studies. This procedure opens up new possibilities in site specific labeling of proteins used for NMR-studies. The HSQC spectra demonstrate that NMR-spectra of partially labeled human ubiquitin can be measured.

OTHER EXAMPLES

synthesized protein	MW kDa	% intact protein	biological active
FABP	14,8	96 %	X
GFB	29,6	85 %	X
CAT	25,6	84 %	X
DHFR	21,5	73 %	X
scAb	27,5		X

FABP - Fatty Acid Binding Protein / GTP - Green Fluorescent Protein
CAT - Chloramphenicol Acetyl Transferase
DHFR - Dihydrofolat Reductase / scAb - Single Chain Antibody

ANALYSIS OF PRODUCTS



PROJECT EVOLUTION

REQUIRED DATA

Before an in-vitro synthesis for a specific protein can be performed the following information is needed:

- Gene sequence of the target protein and / or a plasmid containing the gene together with documentation
- Detailed information about the protein such as
  - Subunit composition
  - Disulfide bridges
  - Solubility
  - Stability
  - Potential toxicity

HOW TO OBTAIN THE PROTEIN

Our aim is to meet each customer's individual expression needs. Since every protein is unique, the procedure for the in-vitro synthesis has to be developed in close collaboration with the customer. After the customer has provided the genetic information of the desired protein the following steps are performed:

- Theoretical evaluation and set-up of an in-vitro expression system
- In-vitro expression of unlabeled protein on a small scale
- In-vitro expression of the protein with a radioactive marker on small scale
- Purification and characterization of the expressed protein on a small scale
- Proposal for the large scale in-vitro expression of SI labeled protein
- Large scale in-vitro expression of stable isotope labeled protein
- Purification and characterization of the expressed SI labeled protein

We guarantee that the information supplied to us will be handled confidentially.

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