PROTEINE – Wozu ?

- **„PROTEIN“: gr. “protos” bedeutet: ´Erstes Element´**
  - **→ Aufbau v. zell-eigenem Eiweiss – Strukturproteine: z.B. Kollagen** (alpha-Helix)
  - **→ Aufbau von Enzymen und Hormonen** — **z.B. Somatotropin, Insulin, Luteinisierendes H.**
  - **→ Für die Reproduktion der Zellsubstanz** — **Enzyme: DNA-Polymerase, RNA-Polymerase, ..**
  - **→ Für Stütz- und Schutzfunktion** — **Z.B.: Keratin (**meist ß-Faltblatt-Strukturen)
  - **→ Wasser-Bindung und Transport:** — **durch Ladung, Aquaporine, ..**
  - **→ Nährstofftransport -** — **Alle Carrier, Ionen-Transport durch Membran**
  - **→ Energiegewinnung,** — **z.B. ATPase**
- **PEPTIDE:**
  - **2 – 10 Aminosäuren: Oligopeptid**
  - **11 – 100 Aminosäuren: Polypeptid**
  - **> 100 Aminosäuren: Protein**
- Richtung der Aminosäurekette:
  - **Aminoanfang (N-terminus)**
  - **und Carboxylende (C-terminus)**
- Peptidbindungen:
  - Spaltung durch Hydrolyse
    - säure-katalytische oder
    - enzymatisch (vergleichbar m. Esterbindung).

8 essentielle Aminosäuren

Herkömmlich: „Biol. Wertigk.: BW“

Kartoffel: **75** > Soja: **72** > Getreide: **50** > Hülsenfrüchte: **45**




Diese 8 AS sind für den Menschen essenziell, d.h. er muss sie über die Nahrung aufnehmen:

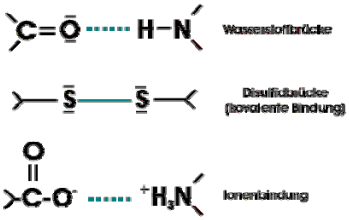
- → Valin
- → Isoleucin
- → Methionin
- → Phenylalanin
- → Leucin
- → Threonin
- → Lysin
- → Tryptophan

<u>Alternative</u> Methode: “Protein digestibility-corrected amino acid score (PDCAAS)”

Protein Quality Evaluation, Report of the Joint FAO/WHO Expert Consultation. Rome: FAO Food and Nutrition Paper No. 51, 1991.

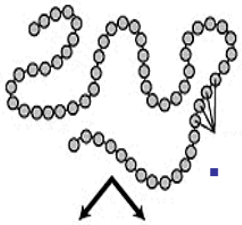
Struktur der Proteine

- **Primärstruktur:** **Reihenfolge der Aminosäuren (AS) in einem Protein (= AS-Sequenz)**
  z.B.: ...-Gly-Leu-Tyr-Ala-Pro-His-...
  - **→ Jedes Protein hat eine durch die DNA eindeutig festgelegte AS-Sequenz**
- **Bereits geringfügige Abweichungen können zum Verlust d. biolog. Aktivität führen**
- **Kombinationen der 20 AS → Nahezu unbegrenzt hohe Anzahl möglicher Proteine!**
- **Physikal-chem. Kräfte zwischen den Kettengliedern bedingen räumliche Anordnung der AS-Kette (Konformation):**
  - **H^+-Brückenbindung**
  - **Kovalente Bindung (Disulfid-Brücken)**
  - **Ionen-Bindung**
  - **hydrophobe Wechselwirkungen**

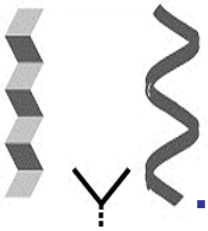


- **Sekundärstruktur:**
  - **Raumanordnung eines Proteins:**
    - **α-Helix *oder***
    - **ß-Faltblattstruktur**
    - **durch Ausbildung von H-Brückenbindungen zw. Peptidbindungen (R-C=O...H-N-R´)**

Peptid – Protein - Struktur

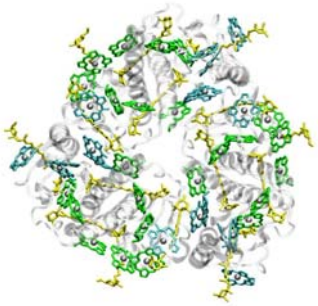


- Primär-Struktur



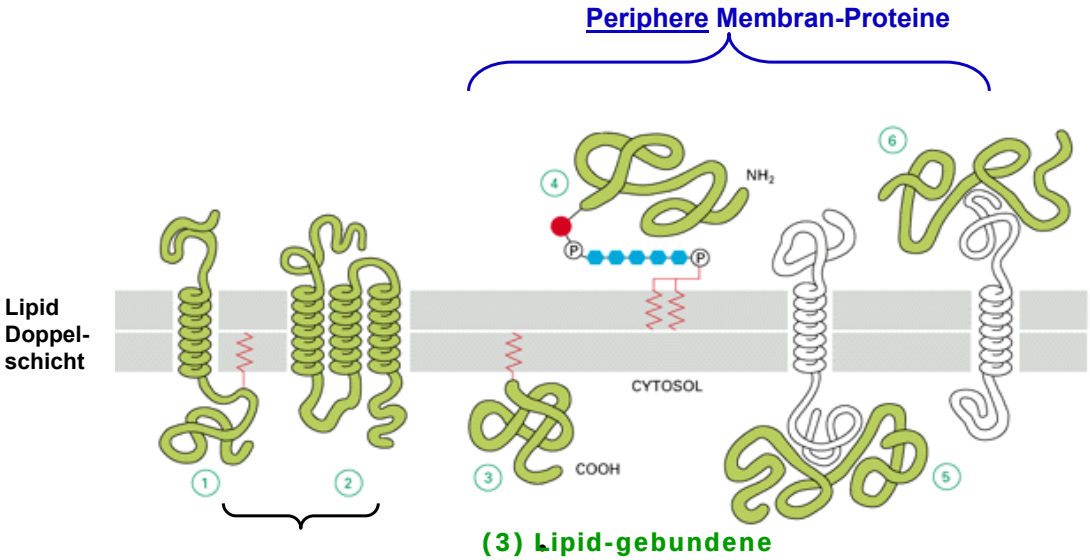
- Sekundär-Struktur

- Teritiär-Struktur
- Quarternär-Struktur



- **Z.B. Light harvesting complex, LHC:**
- **Chlorophyll a (grün), Chlorophyll b (cyan) Carotinoide (gelb), Proteingerüst (grau**

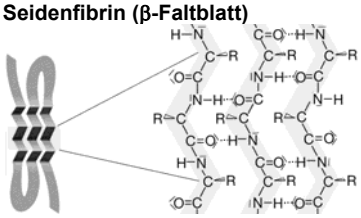
Lage der Proteine



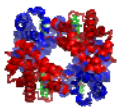
Funktionelle Eigenschaften der Proteine

- **Faserproteine**
  - V.a. Strukturelle Aufgaben:
  - wiederholte Strukturelemente
  - Z. B.: Kollagen (α-Helix),

  - Seidenfibrin (β-Faltblatt)



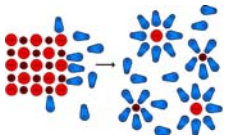
- **Globuläre Proteine**



  - Biol. wirksame Proteine
  - Komplexe Tertiärstrukturen
  - mehrere Arten v Sekundärstrukturen innerhalb derselben Polypeptidkette

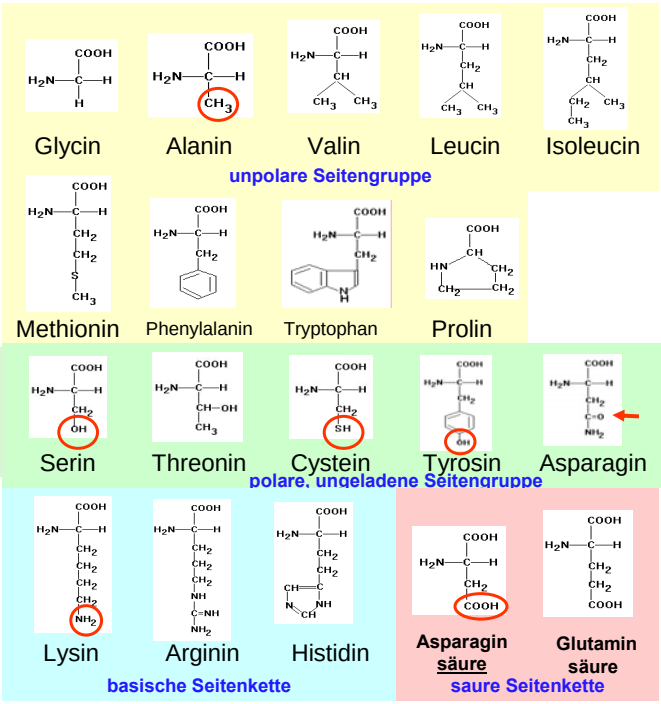
Proteine tragen also bei zu:

- Hydratation
- Löslichkeit
- Viskosität
- Gelbildung
- Texturierung
- → Teig-Stabilität (v.a. Glutene)
- → Schäumung
- → Aroma-Bindung (z.B. Butylamin)
- → Emulgation (z.B. Öl – H_2O)
- → Wechselwirkung mit anderen Lebensmittel-Bestandteilen



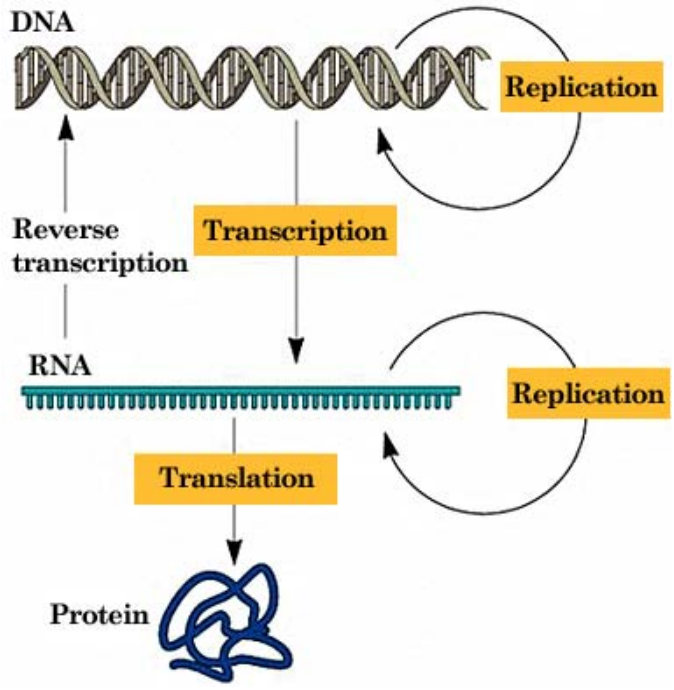
Aminosäuren

- Hydratation
- Löslichkeit
- Viskosität
- Gelbildung
- Texturierung



- Seitengruppen:
  - polar
  - unpolar
  - geladen
  - ungeladen
  - basisch
  - sauer

´Zentrales Dogma´ der Molekularen Biologie



Der genetische Code - Wobble

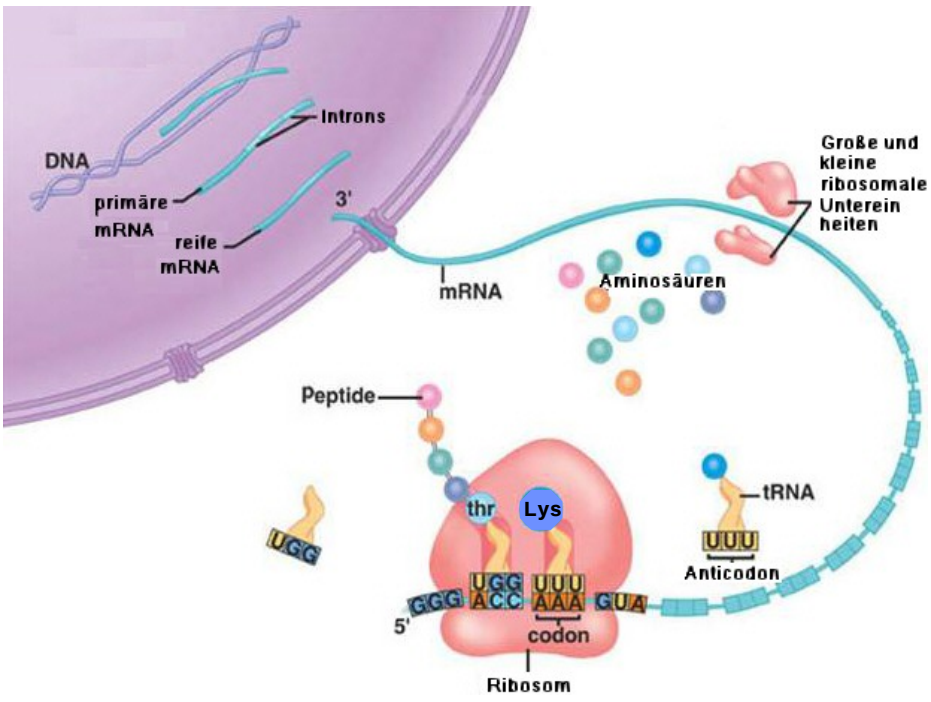
Erste Position

	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Zweite Position

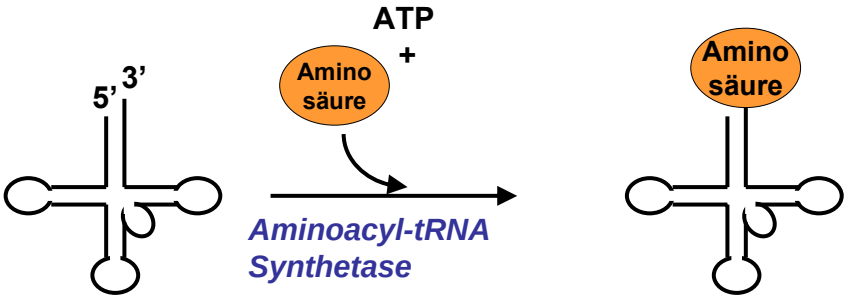
3· (Wobble) Position

ProteinBiosynthese: einfacher Überblick



tRNAs: spezifische Träger der Aminosäuren

- **Aminoacyl-tRNA Synthetasen** transportieren die spezifischen Aminosäuren an die tRNA-Moleküle heran.



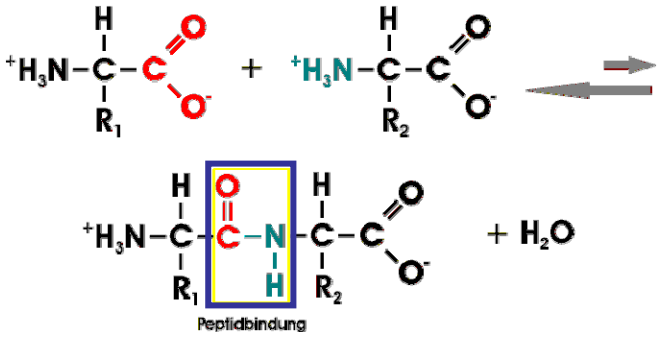
tRNA

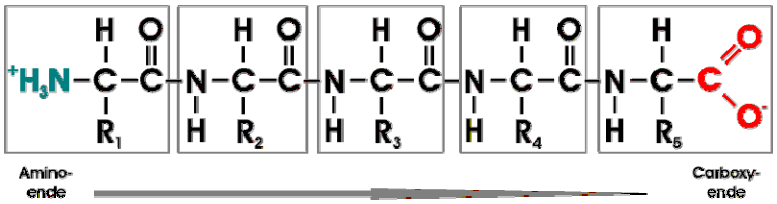
z.B. $tRNA^{Phe}$

Amino-acylierte tRNA

z.B. Phe-$tRNA^{Phe}$

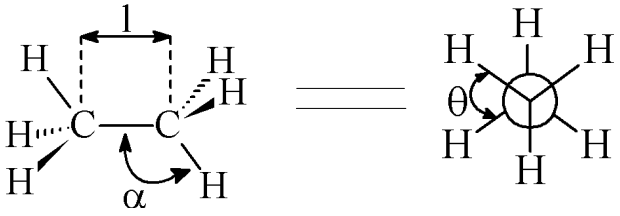
Peptidbindung



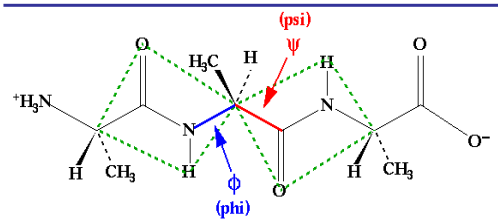


Molekulare Geometrie organischer Verbindungen

- **Die molekulare Geometrie kann durch drei Angaben beschrieben werden:**
  - **Bindungslänge (l)**
  - **Bindungswinkel (α)**
  - **"Diederwinkel" Theta (θ)**



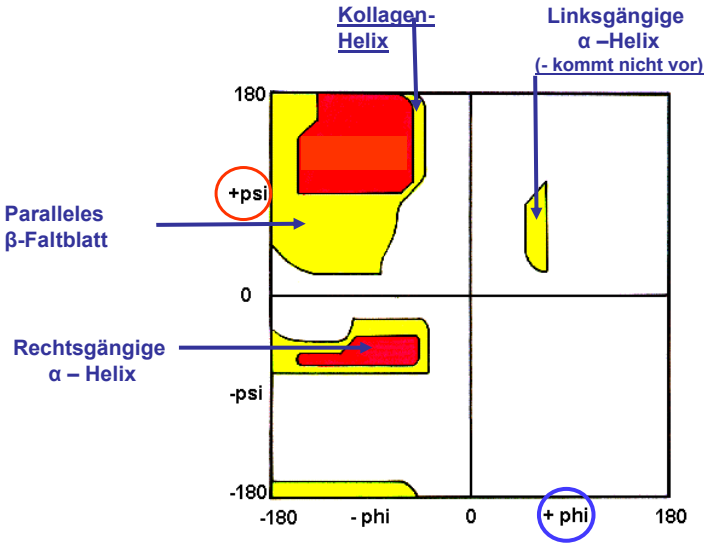
Verdrillung



G. N. Ramachandran and V. Sasiskharan (1968) *Adv. Protein Chem.* 23, 283-437

- **Für jede Aminosäure sind nur zwei Diederwinkel (phi und psi) frei rotierbar.**
- **Die Peptidbindung O=C-N-H ist fast immer planar (gemeinsame Ebene).**
- **Daher kann man die Positionen jeder AS in dem sog. Ramachandran-Plot für die beiden Winkel phi und psi eintragen…**

Sasisekharan-Ramakrisnan-Ramachandran-Diagramm



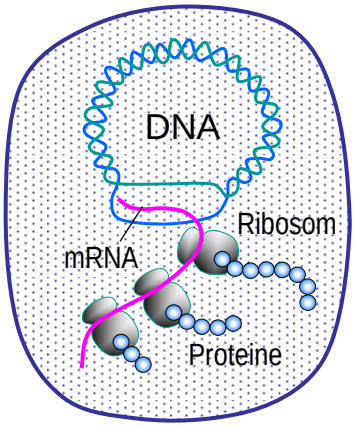
- **Aus sterischen Gründen sind nur bestimmte Winkelkombinationen erlaubt, die im Ramachandran-Plot zu erkennen sind.**
- **Die Struktur einer Polypeptidkette kann durch die Winkel phi (φ)**
- **und psi (ψ) beschrieben werden.**

Ausstattung

Letzte Version der zellulären genetischen Mechanismen

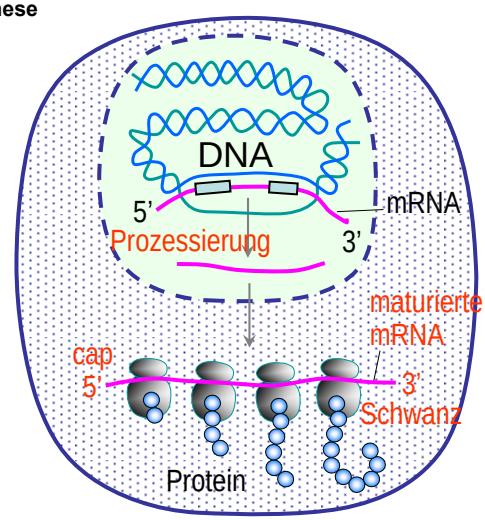
DNA ersetzte RNA und wurde zum Hauptträger der genetischen Information

Die Rolle der RNA wurde v.a. die Protein-Biosynthese



Prokaryont

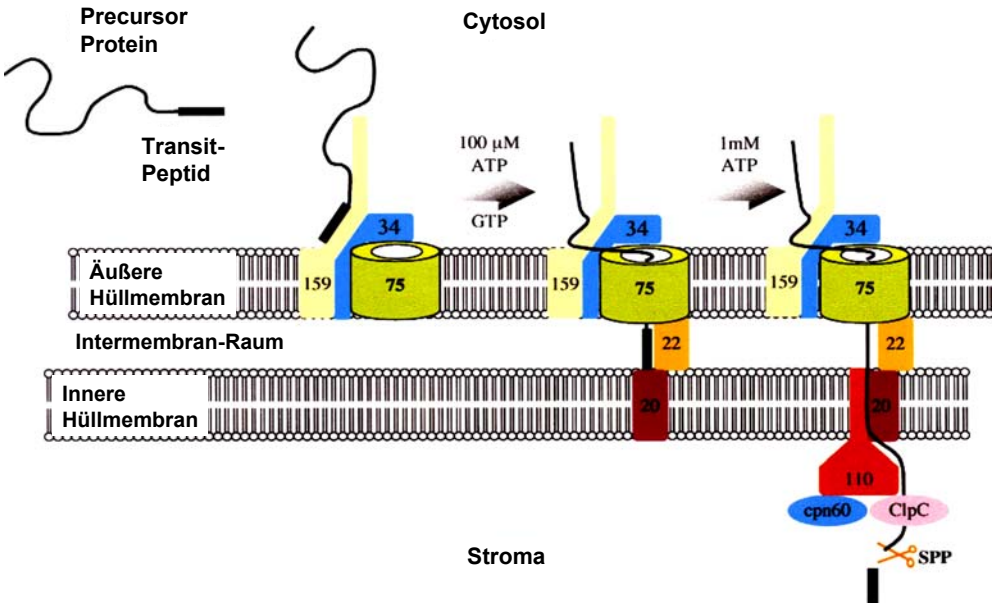
Studentenwohnheim



Eukaryont

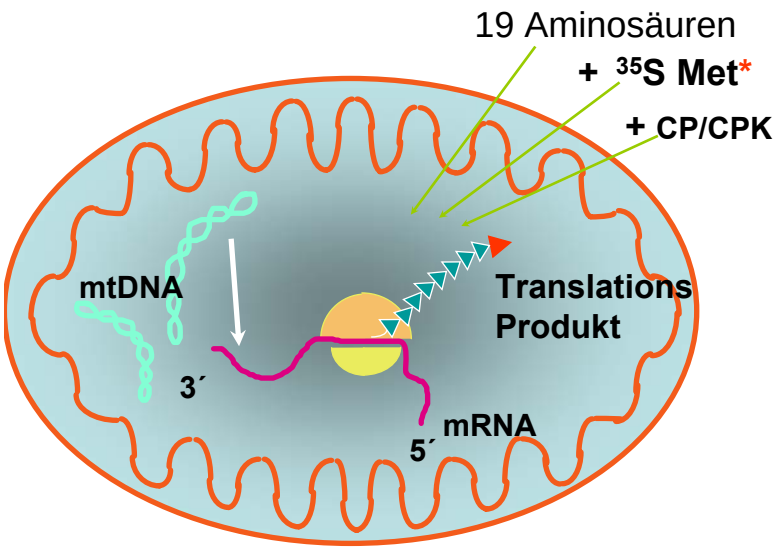
Möbliertes Apartment

Protein-Import -Apparat



Hiltbrunner et al., 2001

In organello Translation

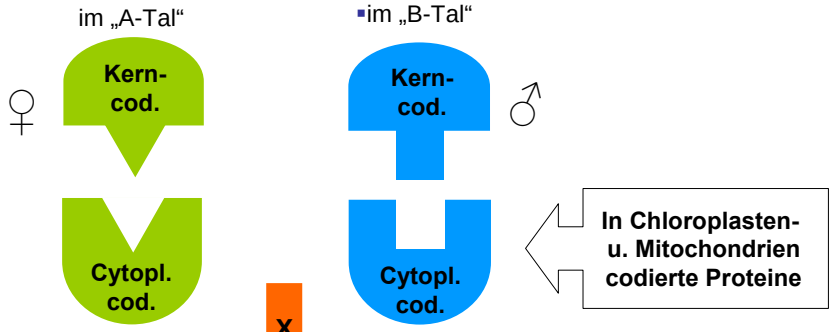


Variabilität zw. Mitochondrien

Am Beispiel
der Kulturkartoffel:
PAGE nach *in-organello*-Translation

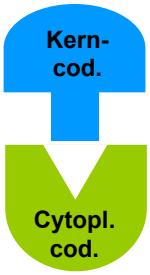
Kern-Cytoplasma-Kompatibität

- 1.: Pflanze gleicher Art: Auseinander-Entwicklung im Laufe der Entwicklungsgeschichte



- 2.: Kreuzung der Pflanzen:
  „A-Tal“ X „B-Tal“

X



- → **CMS**
- → **Chlorosen**
- → **Verzwergung**
- → **Sensitivität für Pilztoxine**
- → **Energiehaushalt generell**

Regulation von Transkription und Translation

Plant Cell Physiol. 46(9): 1462–1471 (2005)
doi:10.1093/pcp/pci157, available online at www.pcp.oupjournals.org
JSPP © 2005

Inducible Trans-activation of Plastid Transgenes: Expression of the *R. eutropha phb* Operon in Transplastomic Tobacco

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[2] *Max-Planck-Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg*
[3] *Institut für Pflanzenbau und Pflanzenzüchtung, Christian-Albrechts-Univ*
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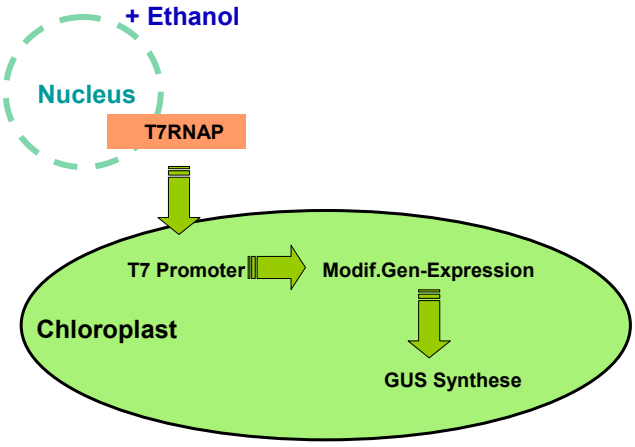
[5] *Ludwig-Maximilians-Universität, Faculty of Biology, Department I, Bota*

Deleterious effects of constitutive transgene expression can occur if gene products are harmful to the transformed plant. Constraints such as growth inhibition and male sterility have been observed in plastid transformants containing the *phb* operon encoding the genes required for the production of the polyester polyhydroxybutyric acid (PHB). In order to induce PHB synthesis in tobacco in a well-timed manner, we have constructed a trans-activation system to regulate transcription of the *phb* operon in plastids. This system consists of a nuclear-located, ethanol-inducible T7RNA polymerase (T7RNAP) which is targeted to plastids harboring the *phb* operon under control of T7 regulatory elements. Following treatment with 5% ethanol, moderate induction of PHB synthesis was found. PHB amounts reached 1,383 ppm in dry weight, and an overall background activity of 171 ppm was measured in uninduced tissues. On the transcriptional level, T7RNAP induction was proven and we found that the *phb* operon is transcribed into at least two mRNAs. Without ethanol induction, development of flowers and fertile seeds was possible. Thus, the main problem of inhibitory transgene expression was solved. Our results show that this inducible trans-activation system could serve as an alternative to constitutive expression of transgenes in the plastome.



Produktion neuer Proteine in Chloroplasten

Transgenic Res
DOI 10.1007/s11248-010-9415-4

ORIGINAL PAPER

Transplastomic expression of a modified human papillomavirus L1 protein leading to the assembly of capsomeres in tobacco: a step towards cost-effective second-generation vaccines

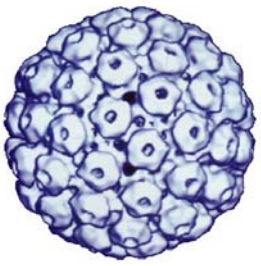
M. Tahir Waheed · Nadja Thönes · Martin Müller · S. Waqas Hassan · N. Mona Razavi · Elke Lössl · Hans-Peter Kaul · Andreas G. Lössl

Received: 22 January 2010 / Accepted: 2 June 2010

Abstract Certain types of human papillomaviruses (HPV) are causatively associated with cervical carcinoma, the second most common cancer in women worldwide. Due to limitations in the availability of currently used virus-like particle (VLP)-based vaccines against HPV to women of developing countries, where most cases of cervical cancer occur, the development of a cost-effective second-generation vaccine is a necessity. Capsomeres have recently been demonstrated to be highly immunogenic and to have a number of advantages as a potential cost-effective alternative to VLP-based HPV vaccines. We have expressed a mutated HPV-16 L1 (L1_2xCysM) gene that retained the ability to assemble L1 protein to capsomeres in tobacco chloroplasts. The recombinant protein yielded up to 1.5% of total soluble protein. The assembly of capsomeres was examined and verified by cesium chloride density gradient centrifugation and sucrose sedimentation analysis. An antigen capture enzyme-linked immunosorbent assay confirmed the formation of capsomeres by using a conformation-specific monoclonal antibody which recognized the conformational epitopes. Transplastomic tobacco plants exhibited normal growth and morphology, but all such lines showed male sterility in the T_0, T_1 and T_2 generations. Taken together, these results indicate the possibility of producing a low-cost capsomere-based vaccine by plastids.

Keywords HPV-16 · L1_2xCysM gene · Capsomeres · Plastids · ELISA · Male sterility

Introduction



- Z.B. Impfstoffe: