The Biotin Carboxylase-Biotin Carboxyl Carrier Protein Complex of *E. coli* Acetyl-CoA Carboxylase

Choi-Rhee et al. 2003

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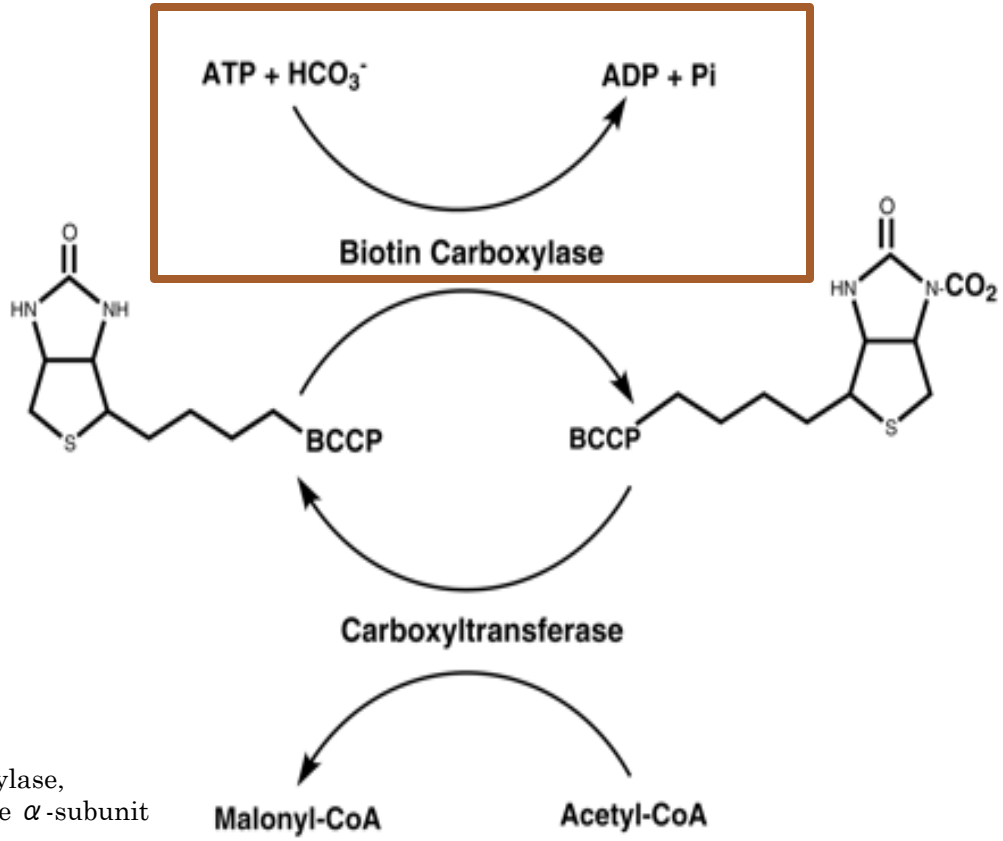
Reaction Mechanism of acetyl-CoA carboxylase (ACCase)



Gene	Protein	Enzyme activity
accA	AccA	Acetyl-CoA carboxylase, carboxyltransferase α-subunit
accB	**AccB**	**Acetyl-CoA carboxylase, carboxy-biotin carrier protein**
accC	**AccC**	**Acetyl-CoA carboxylase, biotin carboxylase**
accD	AccD	Acetyl-CoA carboxylase, carboxyltransferase β –subunit

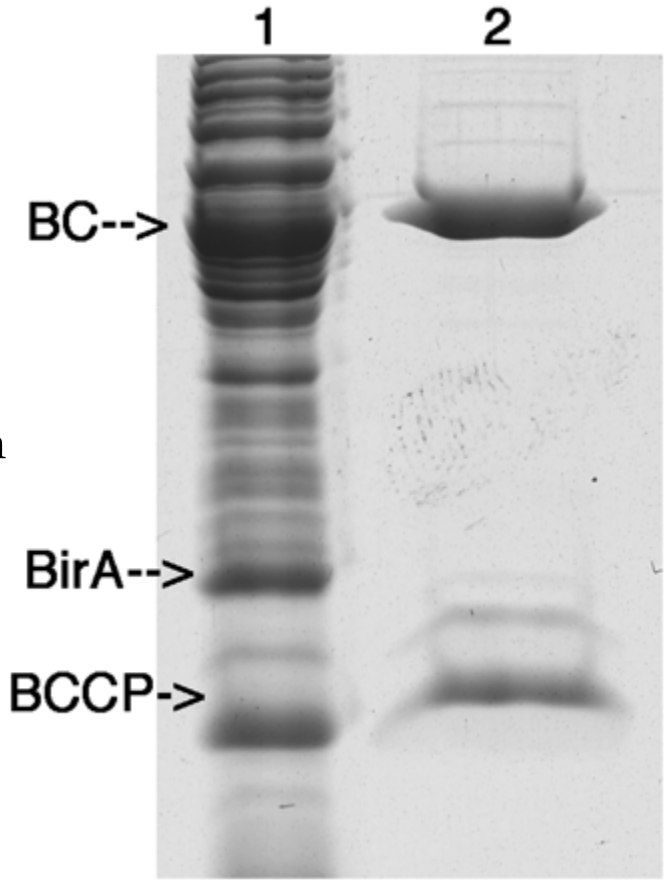
*Cronan et al. 2000

PURIFICATION OF THE BC:BCCP COMPLEX USING A MONOMERIC AVIDIN COLUMN

- BCCP-has a natural affinity tag, biotin moiety
  - Monomeric avidin column
  - Eluted with 2mM biotin

- Overproduced *E. coli* biotin protein ligase (BirA) protein
  - Attaches biotin to lysine 122 of BCCP

FIGURE 1

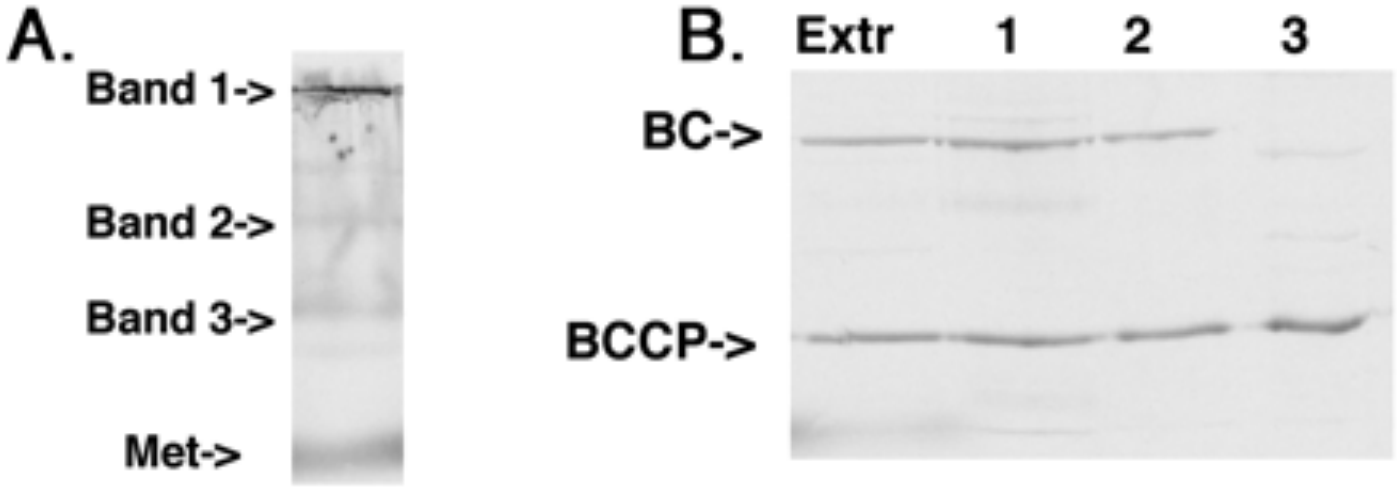


Lane 1: Crude extract
Lane 2: Purified protein

BC:BCCP COMPLEX LABELED WITH L-[^{35}S]METHIONINE

- Question:
  - What is the protein stoichiometry of the BC:BCCP complex, examining BCCP
- Rifampicin added to block protein synthesis of chromosomal genes.
  - L-[^{35}S]methionine
- Purified BC:BCCP complex through monomeric avidin column
  - Native PAGE
  - SDS-PAGE
- Phosphorimaging device

FIGURE 2



BC:BCCP
Lane 1: Crude extract-molar ratio of 1:2
Lane 2: Band 1-molar ratio of 1:1.95
Lane 3: Band 2-molar ratio of 1:1.3
Lane 4: Band 3-only BCCP

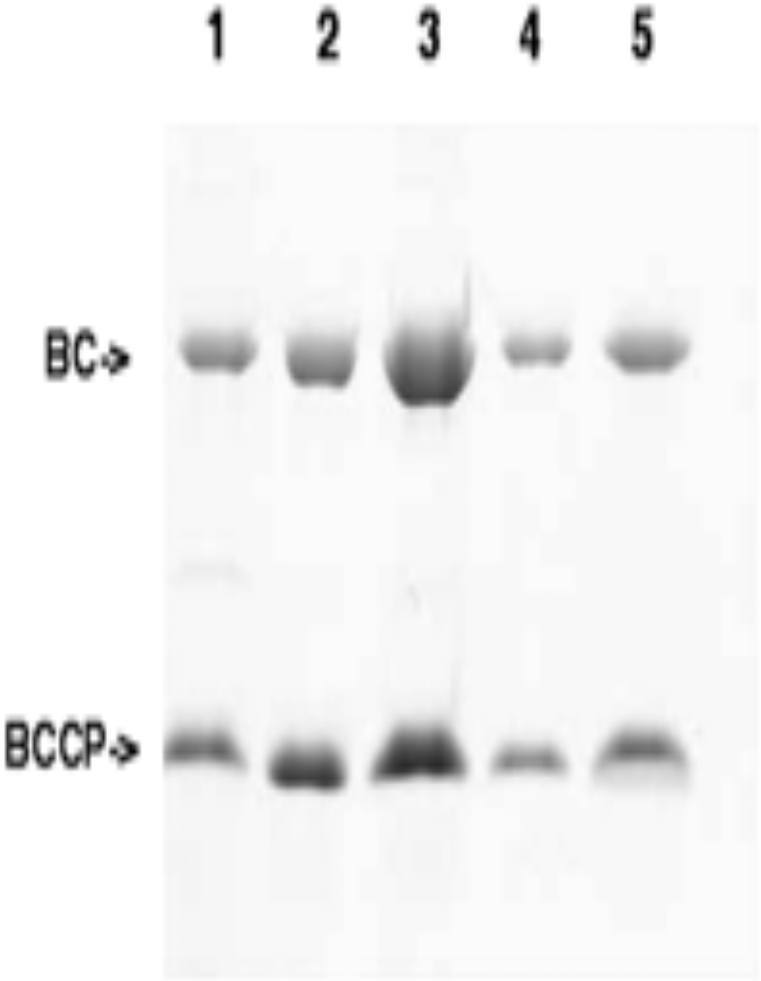
Analysis of BC:BCCP complex by chromatography through immobilized avidin and/or immobilized nickel chelate column

- Question: What is the protein stoichiometry of the BC:BCCP complex, examining BC
- N-terminal His-tag BC protein
- Purified BC:BCCP complex through nickel chelate column
  - Native conditions
- Purified BC:BCCP complex through tetrameric avidin column

FIGURE 3

BC:BCCP

- **Lane 1**: Crude extract-molar ratio 1:2
- **Lane 2:** Avidin column-molar ratio 1:3
- **Lane 3:** Nickel chelate column-molar ratio 1:1.4
- **Lane 4:** (5uL) Eluted from both columns-1:2
- **Lane 5:** (10uL) Eluted from both columns-1:2



Analysis of the purified complex by native PAGE

- Question
  - How many copies of each protein is present in the BC:BCCP complex

- Purified the complex on non-denaturing gels with purified BC as a standard

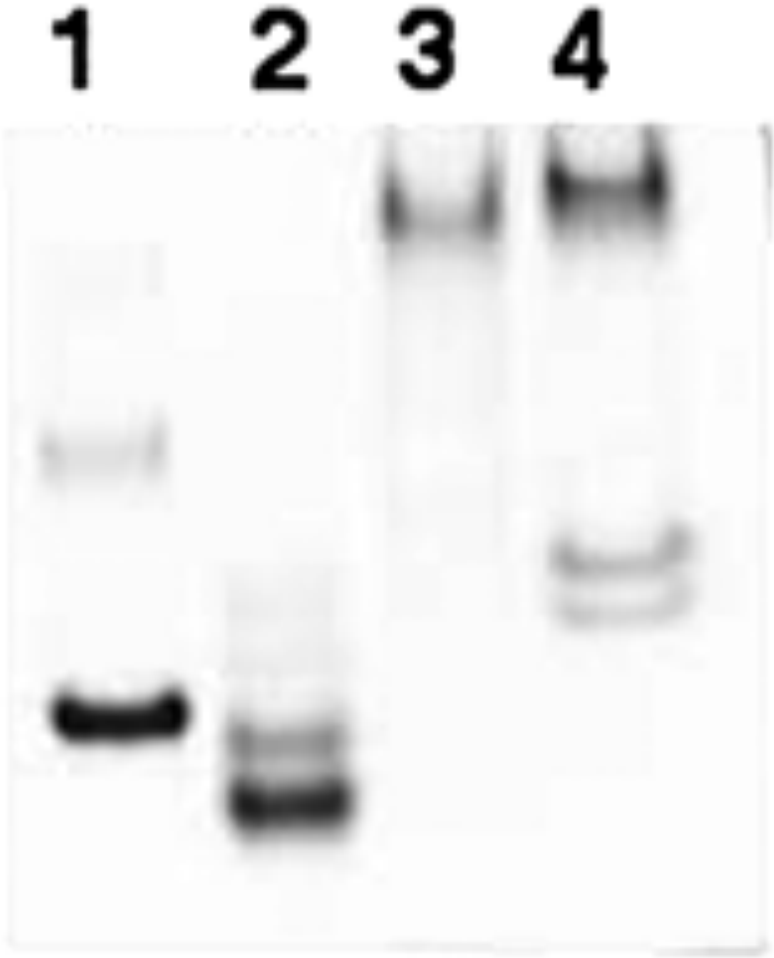
FIGURE 4

Lane 1: Bovine Serum Albumin (67kDa)

Lane 2: Ovalbumin (43kDa)

Lane 3: His-tagged BC

Lane 4: BC:BCCP complex



Effect of deletions within the BCCP N-terminus on formation of the BC:BCCP complex

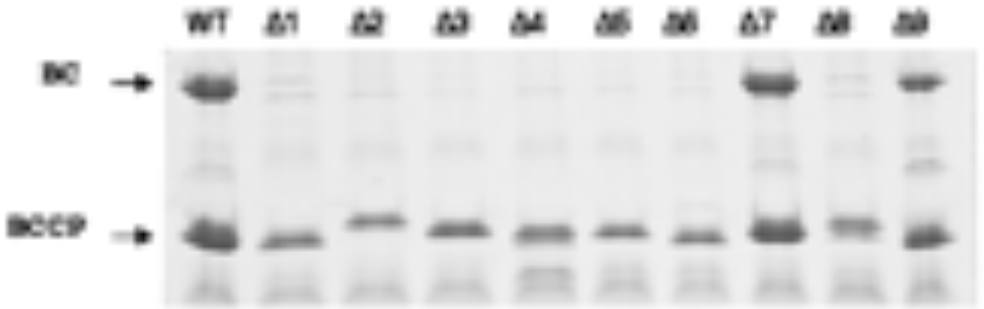
- Question:
  - What is the role of the N-terminal region in BC:BCCP interaction
- Constructed a series of four aa residue deletions within the N-terminal region
- Assayed the ability of the mutated protein to interact with BC

FIGURE 5

A.

	N-Terminal sequence	Complex formation?
WT	MDIRKIKKLIELVEESGISELEISEGEESVRISRAAP	Yes
Δ1	M IRKLIELVEESGISELEISEGEESVRISRAAP	No
Δ2	MDIRK IELVEESGISELEISEGEESVRISRAAP	No
Δ3	MDIRKIKKL EESGISELEISEGEESVRISRAAP	No
Δ4	MDIRKIKKLIELV ISELEISEGEESVRISRAAP	No
Δ5	MDIRKIKKLIELVEESG EISEGEESVRISRAAP	No
Δ6	MDIRKIKKLIELVEESGISEL GEESVRISRAAP	No
Δ7	MDIRKIKKLIELVEESGISELEISE VRISRAAP	Yes
Δ8	MDIRKIKKLIELVEESGISELEISEGEES RAAP	No
Δ9	MDIRKIKKLIELVEESGISELEISEGEESVRIS	Yes

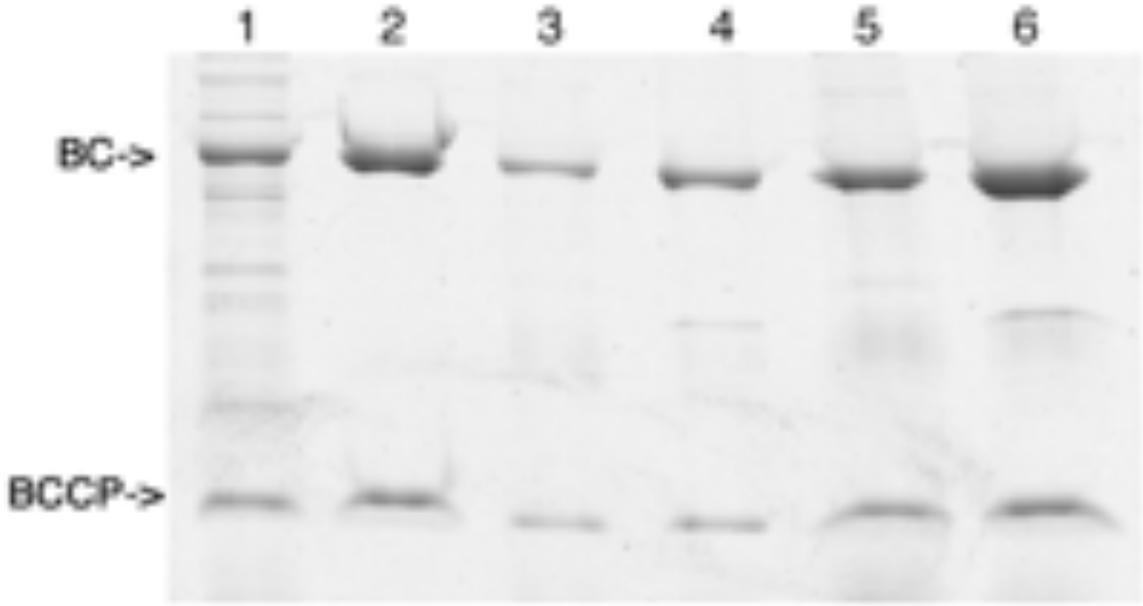
B.



BIOTINYLATION OF THE BC:BCCP COMPLEX *IN VITRO*

- Question: Does biotinylation of BCCP occur before or after assembly of the protein into the BC:BCCP complex?
- Purified an under-biotinylated BC:BCCP complex on a nickel chelate column
- Biotinylated *in vitro* with purified BirA, ATP, and biotin
- Purified the complex using an avidin column

FIGURE 7



Lane 1: Crude extract
Lane 2: Nickel chelate-purified complex
Lanes 3 and 5: Samples of the complex that were not biotinylated *in vitro*
Lanes 4 and 6: Samples of the complex that had been biotinylated *in vitro*.