Identification of 23 Complementation Groups Required of Post-translational Events in the Yeast Secretory Pathway

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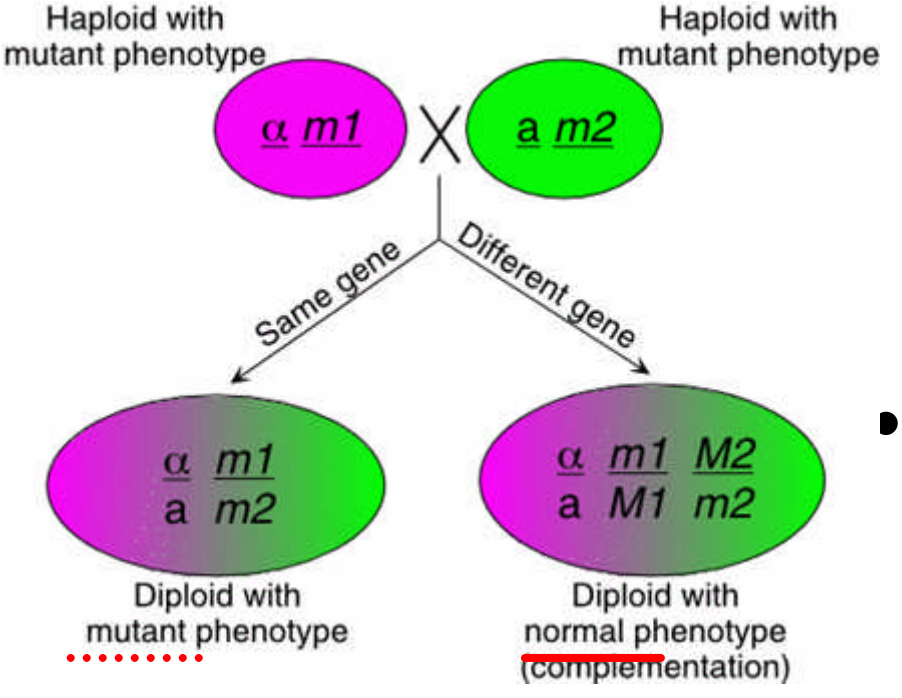
Cell, **21**:205-215

Present by Liang

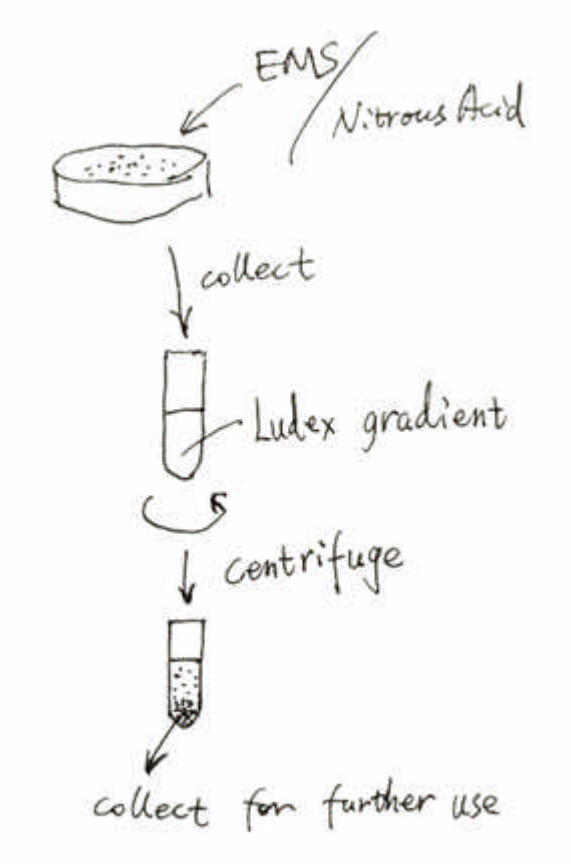
Background



- **Goal:** study secretory process
- **Basic:** complementation test

http://saturn.roswellpark.org/cmb/huberman/BIR572/Fig7YeastGenetics.html

Research Strategy



1. Mutation
2. **Selection**
3. Comp. Test
4. Identify
5. Discussion

Density Enrichment

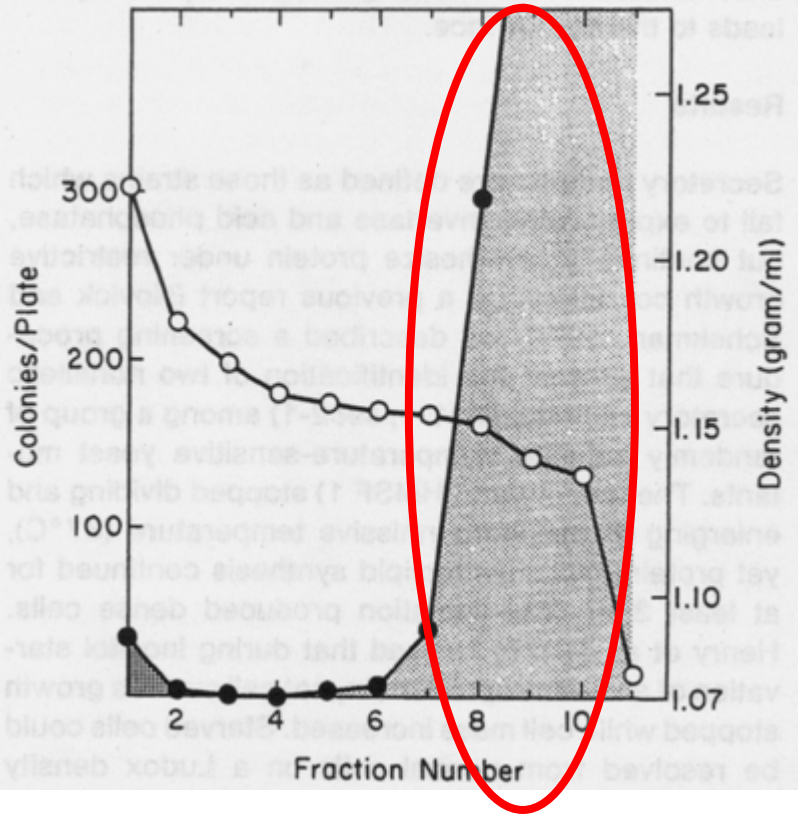


Table 1. Comparison of Screening Procedure with and without Density Enrichment

	Without Enrichment		With Enrichment	
Screening Stage	Colonies	%	Colonies	%
(1) Colonies tested	5,600	100	18,500	100
(2) TS mutants	291	5.2	2,830	15
(3) TS phosphatase secretion	63	1.1	980	5
(4) TS invertase secretion	16	.29	485	2.6
(5) TS invertase accumulation	2	.04	188	1.0

Complementation Test

Table 2. Distribution of Mutants in the *sec*A Complementation Groups: EMS versus Nitrous Acid

	EMS		Nitrous Acid	
sec	Isolates	%	Isolates	%
1	8	11	4	3
2	28	39	41	35
3	3	4	0	0
4	7	10	2	2
5	10	14	16	14
6	3	4	3	3
7	1	1	3	3
8	6	8	4	3
9	3	4	4	3
Total:	72	98%	116	100%

25C, Invertase Accumulation

Table 3. Invertase Secretion and Accumulation by the *sec* Mutants

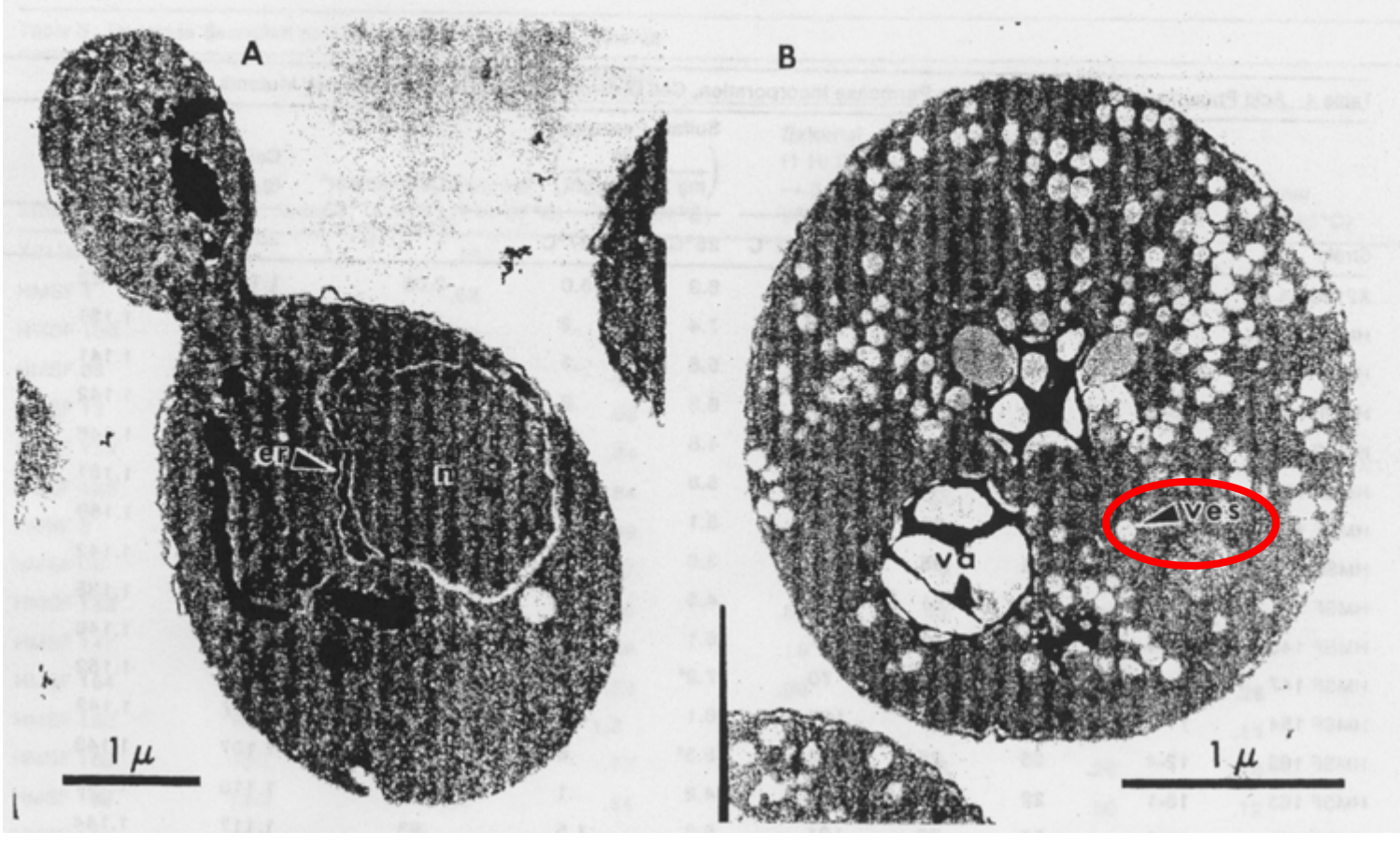
Strain	*sec* Group	Units/mg Dry Weight: External[a] (1 Hr 37°C)	Internal (1 Hr 37°C)	External (1 Hr 37°C → 3 Hr 25°C)	% Release[b]	External (1 Hr 25°C)	Internal (1 Hr 25°C)
X2180-1A		.38	.08	.33	0	.34	.14
HMSF 1	1-1	.02	.61	.28	43	.29	.15
HMSF 106	2-56	.03	.87	.36	38	.24	.18
HMSF 68	3-2	.02	.31	.05	9	.31	.22
HMSF 13	4-2	.05	.63	.13	11	.32	.30
HMSF 134	5-24	.03	.84	.08	6	.39	.17
HMSF 136	6-4	.03	.84	.46	52	.36	.14
HMSF 6	7-1	.04	.39	.10	16	.42	.29
HMSF 95	8-6	.03	.57	.07	7	.37	.22
HMSF 143	9-4	.09	1.05	.53	42	.20	.28
HMSF 147	10-2	.03	.68	.15	18	.31	.17
HMSF 154	11-7	.40	.53	.59	35	.56	.26
HMSF 162	12-4	.04	1.3	.90	64	.22	.11
HMSF 163	13-1	.19	.77	.64	58	.28	.14
HMSF 169	14-3	.07	.64				

25C, Acid Phosphatase & Sulfate Permease Defect

Table 4. Acid Phosphatase Secretion, Sulfate Permease Incorporation, Cell Division and Cell Density of the *sec* Mutants

Strain	*sec*	Acid Phosphatase[a] (Units/ml)			Sulfate Permease[b] (Units / mg Dry Weight)		Cell Number[c] (2 Hr 37°C / 0 Hr)	Cell Density[d] (g/ml)	
		2.5 Hr	5 Hr 37°C	5 Hr 25°C	25°C	37°C		25°C	37°C
X2180 1A		27	193	174	6.3	5.0	2.03	1.110	1.122
HMSF 1	1-1	27	28	147	7.4	.2	1.10	1.113	1.161
HMSF 106	2-56	50	48	170	5.8	.1	.92	1.109	1.141
HMSF 68	3-2	55	82	411	6.8	.3	1.10	1.109	1.142
HMSF 13	4-4	25	29	202	4.8	.2	1.07	1.116	1.146
HMSF 134	5-24	27	31	177	5.8	.4	1.04	1.110	1.161
HMSF 136	6-4	25	27	178	5.1	.1	1.20	1.111	1.159
HMSF 6	7-1	86	95	320	3.6	.03	.91	1.103	1.142
HMSF 16	8-1	44	81	223	4.5	.9	1.11	1.103	1.135
HMSF 143	9-4	30	30	177	6.1	.1	1.04	1.111	1.146
HMSF 147	10-2	25	47	70	7.2[e]	.03	.92	1.117	1.152
HMSF 154	11-7	16	15	107	6.1	2.0	1.48	1.117	1.142
HMSF 162	12-4	25	25	89	5.3[e]	.65	1.05	1.107	1.143
HMSF 163	13-1	22	18	154	4.2	.1	1.01	1.113	1.141
HMSF 169	14-3	25	26	121	5.3	1.5	.93	1.117	1.144
							1.15	1.117	1.159

TSEM of Cells, 25C v.s. 37C



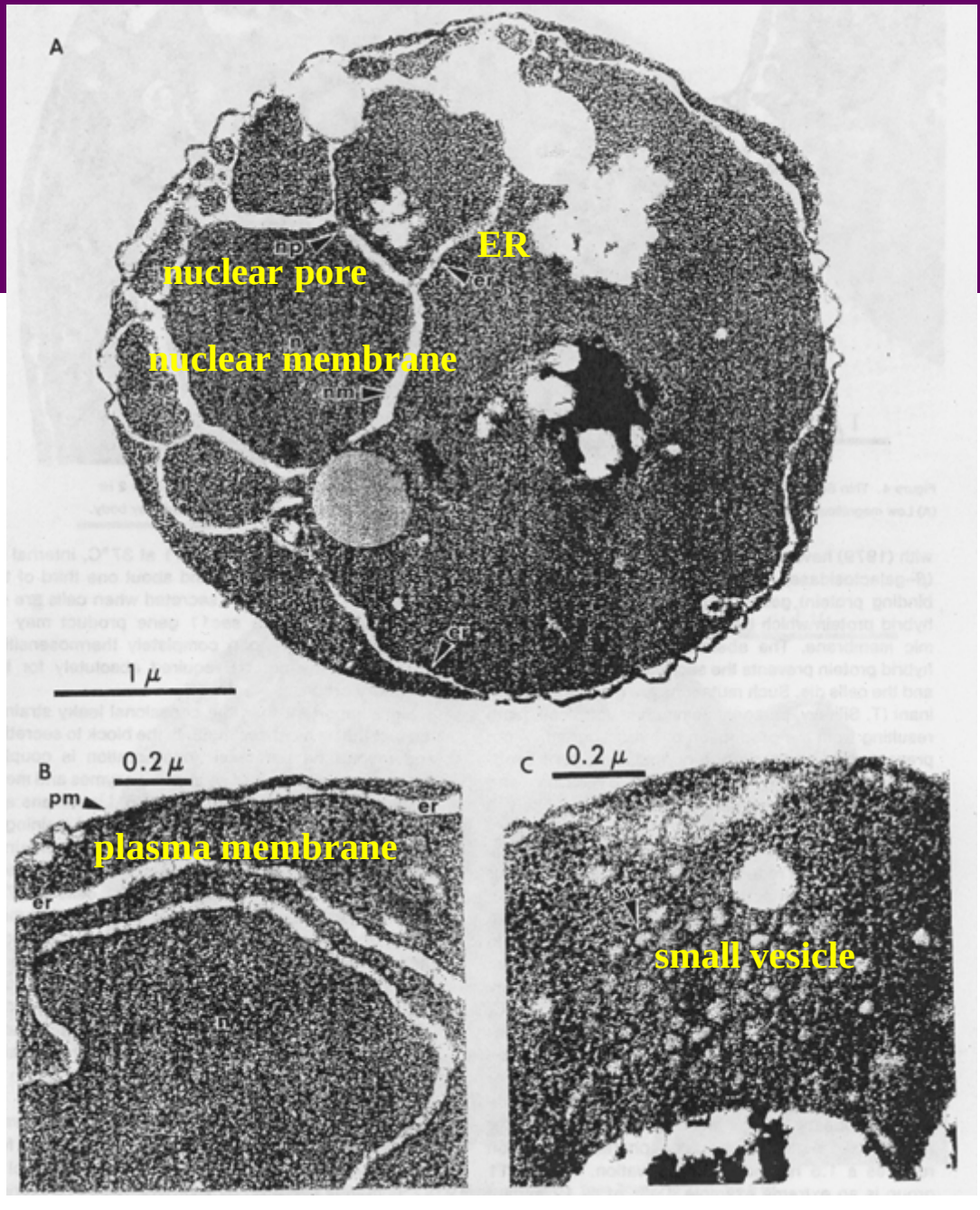
Organelles Accumulation

Table 5. Organelles Accumulated in the *sec* Strains

Strain (HMSF)	*sec*	Structure(s)
1	1-1	vesicles, Berkeley bodies
47	2-7	vesicles
3	3-1	vesicles
13	4-2	vesicles
81	5-8	vesicles
12	6-1	vesicles
6	7-1, -2	Berkeley bodies
93	8-4	vesicles
89	9-3	vesicles, Berkeley bodies
147	10-2	vesicles
154	11-7	
162	12-4	ER
163	13-1	ER

Accumulated Structures:

- Vesicle
- Berkeley Body
- ER
- Small Vesicle


A
np
ER
nuclear pore
er
nuclear membrane
nm
er
1 μ
B
0.2 μ
pm
er
plasma membrane
er
n
C
0.2 μ
sv
small vesicle

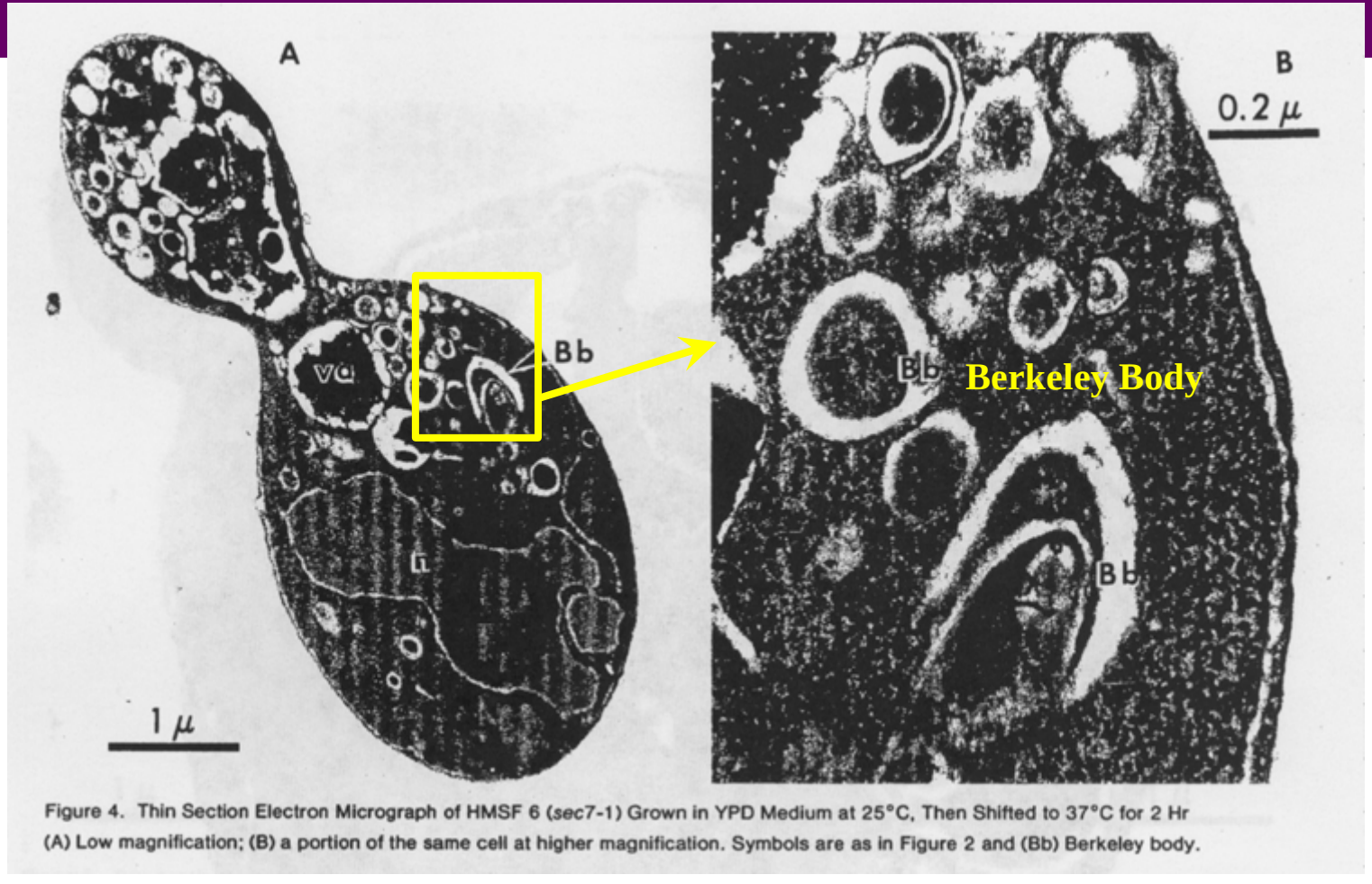
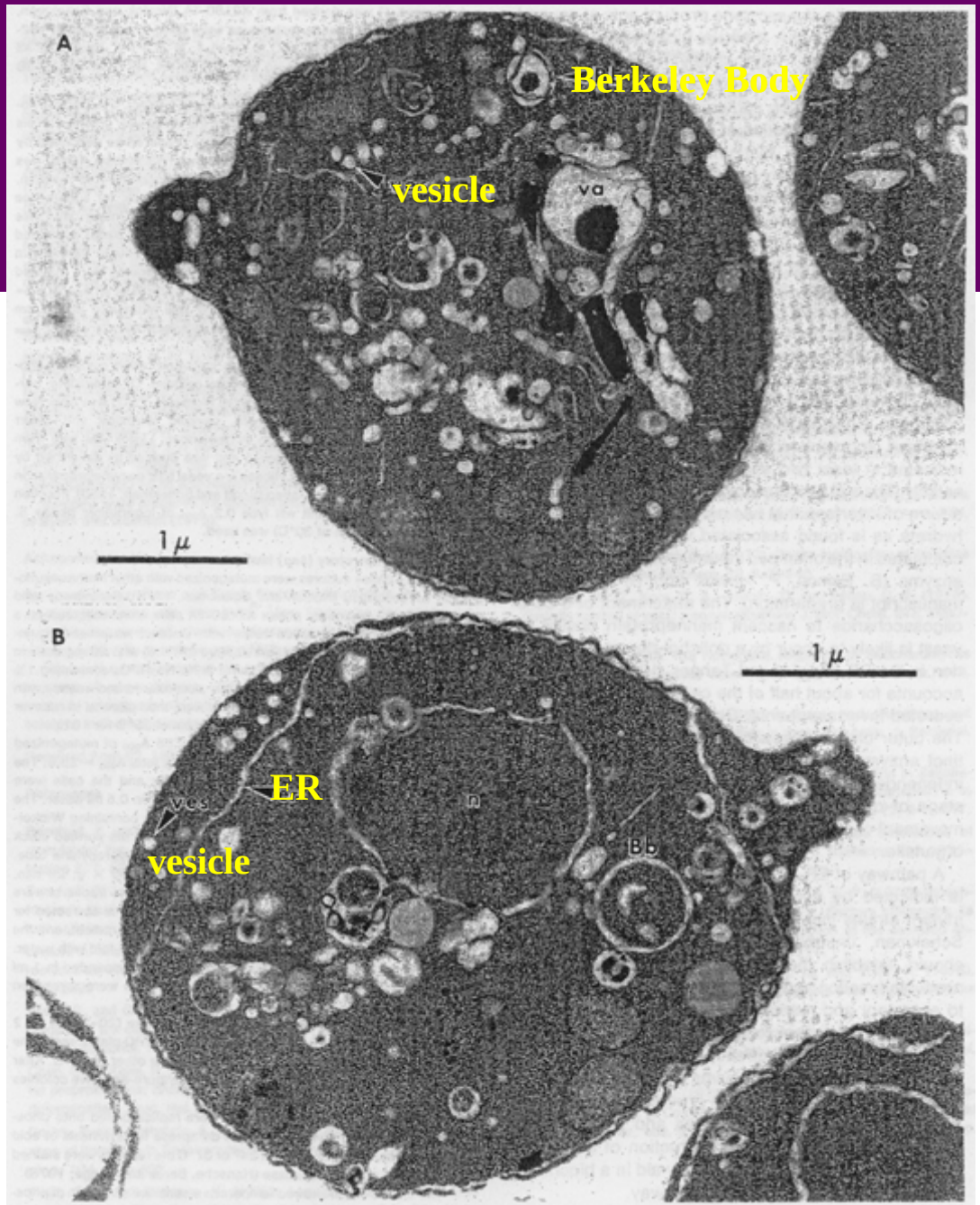


Figure 4. Thin Section Electron Micrograph of HMSF 6 (*sec*7-1) Grown in YPD Medium at 25°C, Then Shifted to 37°C for 2 Hr (A) Low magnification; (B) a portion of the same cell at higher magnification. Symbols are as in Figure 2 and (Bb) Berkeley body.


A
Berkeley Body
vesicle
va
1 μ
B
1 μ
ER
Ves
vesicle
n
Bb

Conclusion

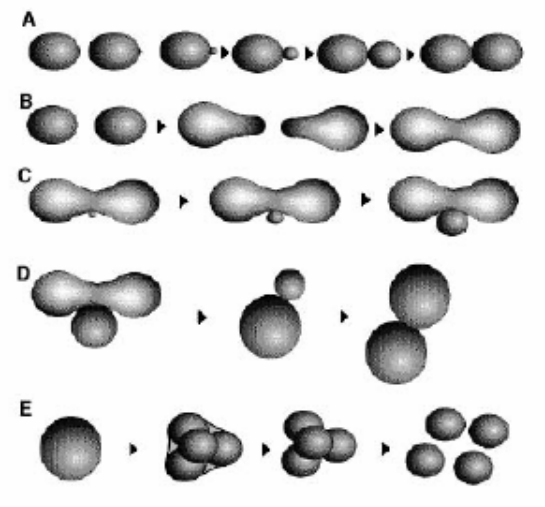
- Ludox Density Gradient accumulated secretion mutants.
- At least 23 genes associate with yeast secretory pathway.
- Membrane-enclosed organelles take part in the secretory pathway:
  ER => Bbs => vesicles => cell surface

Disscussion

- Eliminate some mutants
- TSEM artifact
- One comp. group <> one gene

Appendix

- Yeast Introduction. Cells of the **alpha** mating type are grown overnight on agar medium, a high concentration of the pheromone accumulates in the agar surrounding the growth. Then if cells of the **a** mating type are placed on this agar, they begin to undergo the "shmoo" transformation within a couple of hours.



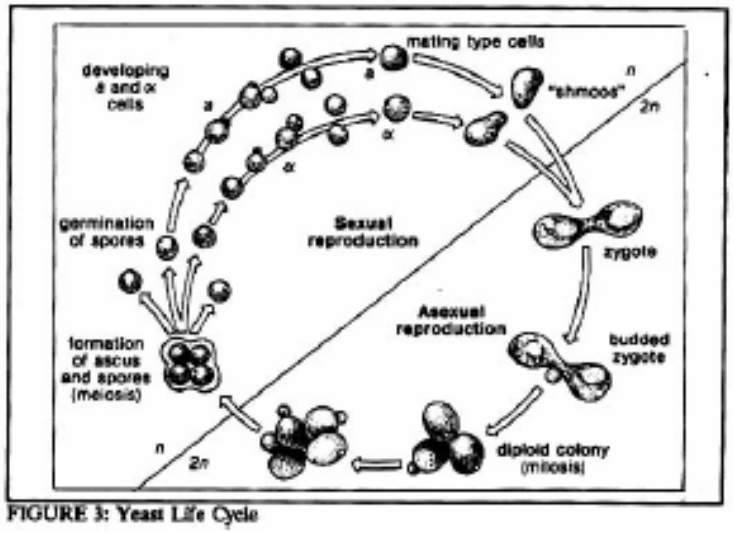


FIGURE 3: Yeast Life Cycle

people.uis.edu/rmosh1/Yeastintromaterial02.pdf