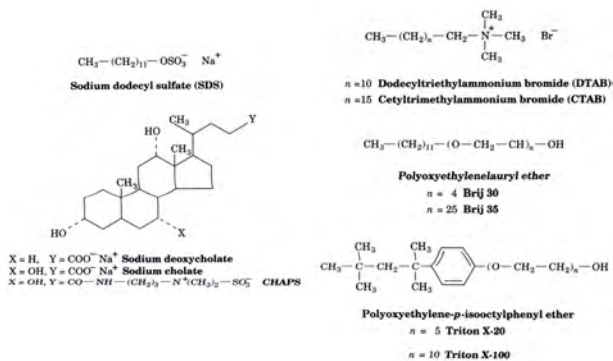


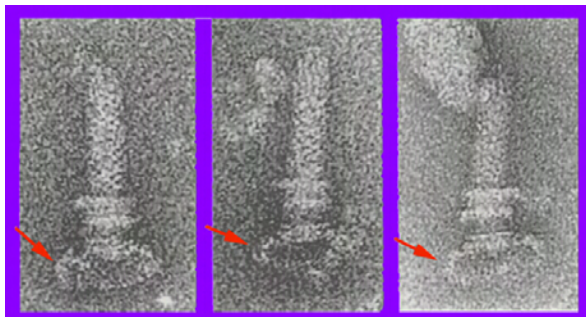
# べん毛の単離プロトコール

- 1) 菌体の回収 (低速遠心)
- 2) ショ糖を含む緩衝液にいれる
- 3) リゾチーム処理+ EDTA
- 4) トリトンX-100処理
- 5) MgSO<sub>4</sub>+DNase
- 6) EDTA
- 7) 低速遠心
- 8) 10万gで遠心
- 9) 沈殿をバッファーに懸濁

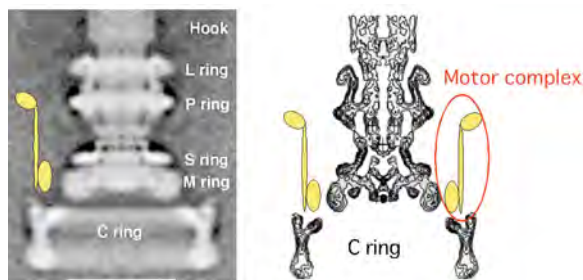
## 生化学で用いられる界面活性剤



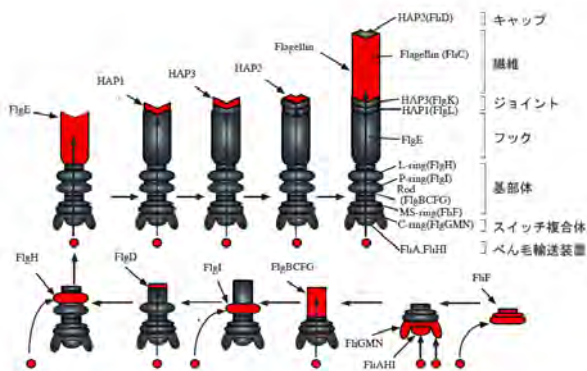
Electron micrographs of hook-basal body and C-ring structure (red arrows)



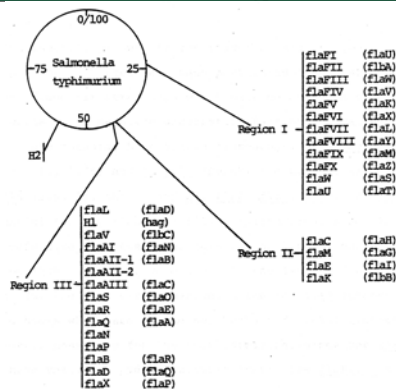
## Motor Structure of flagella



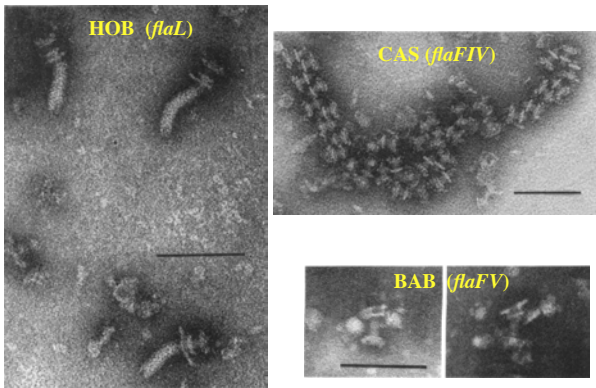
## べん毛形成過程のモデル



## Chromosomal map of the genes essential for flagellar formation in *Salmonella typhimurium*.

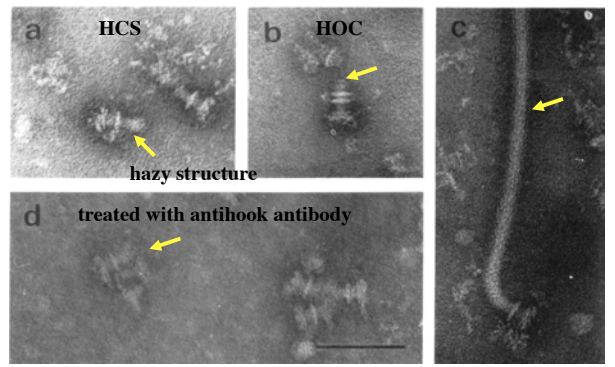


### Flagellar partial structures I



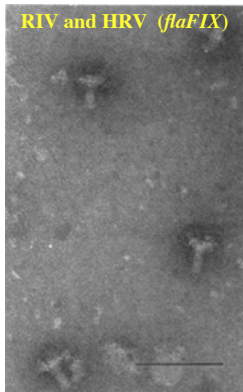
Suzuki et al., J. Bacteriol. (1978)

### Flagellar partial structures II: from a *flaFVIII* mutant



Suzuki et al., J. Bacteriol. (1978)

### Flagellar partial structures III



Suzuki et al., J. Bacteriol. (1978)

Frequencies of flagellar structures detected in nonflagellate mutants\*

| Determination                   | Structural entity |     |     |        |     |     |     |        |     |   |
|---------------------------------|-------------------|-----|-----|--------|-----|-----|-----|--------|-----|---|
|                                 | IF                | HOB | BAB | CAS    | RIV | RCT | HCS | HRV    | HOC |   |
| Flagellate parent               | +++               | +   | +   | +      | +   | +   | +   | +      | +   | + |
| H <sup>1</sup> , H <sup>2</sup> | -                 | ++  | +   | +      | +   | +   | -   | -      | -   | - |
| <i>flaL</i>                     | -                 | ++  | +   | +      | +   | +   | -   | -      | -   | - |
| <i>flaU</i>                     | -                 | ++  | +   | +      | +   | +   | -   | -      | -   | - |
| <i>flaR</i>                     | -                 | ++  | +   | +      | +   | +   | -   | -      | -   | - |
| <i>flaFV</i>                    | -                 | -   | +   | +      | ++  | ++  | -   | -      | -   | - |
| <i>flaFVIII</i>                 | -                 | -   | -   | +      | +   | -   | ++  | + or - | +   | + |
| <i>flaFI</i>                    | -                 | -   | -   | -      | ++  | -   | -   | ++     | -   | - |
| <i>flaFIX</i>                   | -                 | -   | -   | -      | ++  | -   | -   | ++     | -   | - |
| <i>flaFIV</i>                   | -                 | -   | -   | ++     | +   | -   | -   | -      | -   | - |
| <i>flaAI</i>                    | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaAII</i>                   | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| ( <i>motC</i> )                 | ++                | +   | +   | + or - | +   | -   | -   | -      | -   | - |
| <i>flaAIII</i>                  | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaB</i>                     | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaC</i>                     | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaD</i>                     | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaE</i>                     | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaFII</i>                   | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaFIII</i>                  | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaFVI</i>                   | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaFVII</i>                  | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaFX</i>                    | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaK</i>                     | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |
| <i>flaM</i>                     | -                 | -   | -   | -      | -   | -   | -   | -      | -   | - |

\*The frequencies of IF and flagellar basal structures detected in fraction BMII of flagellate parents and nonflagellate mutants are shown.

+++ The count of each structural entity from 1/5 to 5x the count of IF in its flagellate parent.

++ The count of each structural entity from 1/10 to 1/5 the count of IF in its flagellate parent.

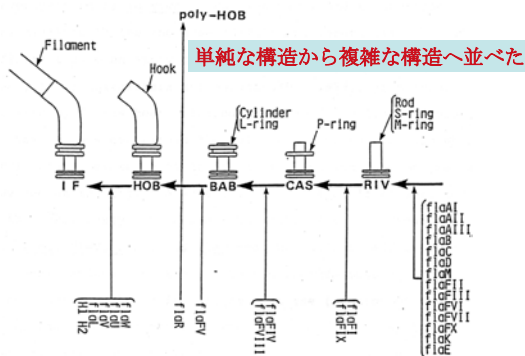
+ The count of each structural entity less than 1/10 the count of IF in its flagellate parent.

- Polyoxyhook basal body complexes were detected.

Paralyzed flagella, which were not discriminated morphologically from the IF of their flagellate parent.

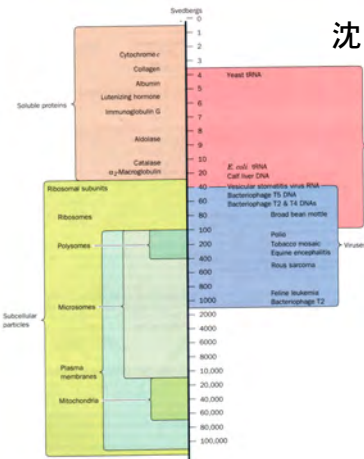
Suzuki et al., J. Bacteriol. (1978)

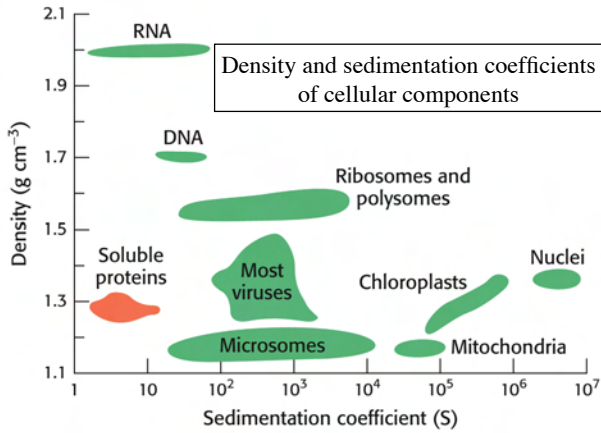
### Stepwise process of flagellar morphogenesis in *Salmonella* inferred from the flagellar structures detected on nonflagellate mutants.



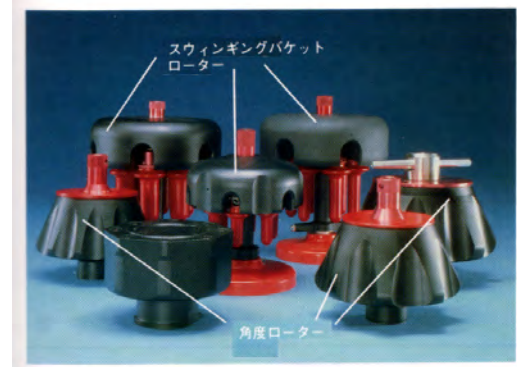
単純な構造から複雑な構造へ並べた

### 沈降係数





### 超遠心ローター



### 超遠心機



型式: CP100MX  
 最高回転速度(rpm): 100,000  
 最大遠心加速度(xg): 803,000  
 回転制御精度(rpm): ±10  
 加速減速時間: 0~100,000rpm:5分  
 温度制御精度: 表示: ±0.5℃  
 真空方式:  
 油回転真空ポンプ+油拡散真空ポンプ  
 到達圧力0.13Pa以下  
 駆動部保証: 完全10年間  
 冷却方式:  
 フロンレス、サーモモジュール冷却システム  
 表示  
 大きさ(mm): (W)790×(D)690×(H)1,000  
 質量(kg): 400  
 標準価格(円):

### 遠心分離 I

#### 遠心力

角速度 (rad · s<sup>-1</sup>) = ω = dθ/dt  
 加速度 = α = r ω<sup>2</sup> 半径 = r  
 加速度 g = 9.8 m/s<sup>2</sup>  
 r = 10 cm    6,000 rpm ⇒ 0.1 · (2π · 100)<sup>2</sup> = 39,438 m/s<sup>2</sup> = 4,024 g  
 30,000 rpm ⇒ 0.1 · (2π · 500)<sup>2</sup> = 985,960 m/s<sup>2</sup> = 100,608 g

沈降力  $F_s = m\omega^2 r - V\rho\omega^2 r$      $V_p = \text{体積}$   
 $F_f = v f$      $v = \text{粒子の沈降速度}$   
 $f = \text{摩擦係数}$   
 $\rho = \text{溶液の密度}$   
 $m = \text{質量}$

粒子の沈降速度は沈降力と摩擦力が釣り合うまで加速する  
 $m = M(\text{分子量}) / N(\text{アボガドロ数})$

従って  $m\omega^2 r - V\rho\omega^2 r = v f$

$V_p = \bar{V} m = \frac{\bar{V} M}{N}$

$\bar{V} = \text{偏比容と密度の逆数}$

1 g の粒子を無限大溶液に溶かしたときの溶液増加

20 °C の DW に蛋白質を溶かしたとき ⇒ 約 0.73 cm<sup>3</sup> g<sup>-1</sup>

### 遠心分離 II

$V_p = \bar{V} \cdot m$ ;  $\bar{V} = \text{偏比容と密度の逆数}$

$V_p = \bar{V} m = \frac{\bar{V} M}{N} \rightarrow v f = \frac{M(1 - \bar{V}\rho)\omega^2 r}{N}$

沈降係数  $s$  を定義する  $10^{-13} s = 1S(\text{ストベリ})$  として表す

$s = \frac{v}{\omega^2 r} = \frac{1}{\omega^2} \left( \frac{d \ln r}{dt} \right) = \frac{M(1 - \bar{V}\rho)}{Nf}$

加速度に対する粒子の沈降速度

半径  $r$  の粒子の  $f$  (摩擦係数) はストークの式で計算される

$f = 6\pi\eta r_p$      $\eta = \text{粘度}$

$f$  と  $f_0$  (最小摩擦係数: 水和していない球体) を求めることで分子形が推定出来る

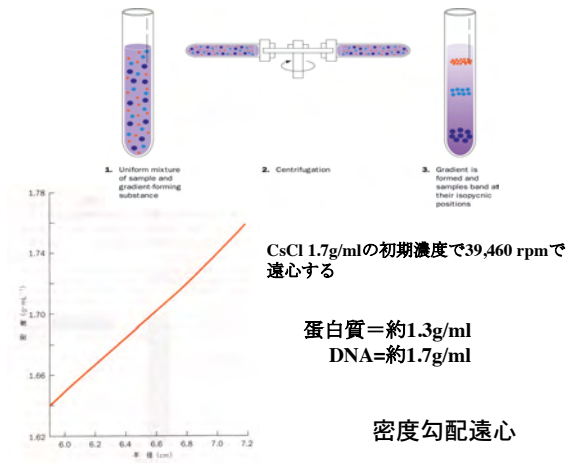
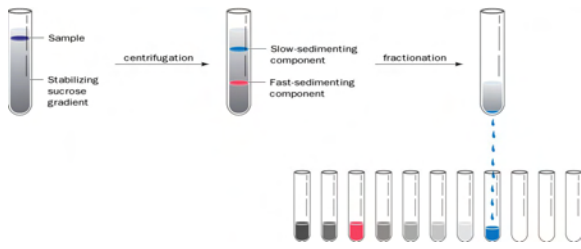
Table 6-5 Physical Constants of Some Proteins.

| Protein                                     | Molecular Mass (kD) | Partial Specific Volume, $V_{20,w}$ (cm <sup>3</sup> · g <sup>-1</sup> ) | Sedimentation Coefficient, $s_{20,w}$ (S) | Frictional Ratio, $f/f_0$ |
|---|---------------------|--|---|---------------------------|
| Lipase (milk)                               | 6.7                 | 0.714  | 1.14                                      | 1.190                     |
| Ribonuclease A (bovine pancreas)            | 12.6                | 0.707  | 2.00                                      | 1.066                     |
| Cytochrome c (bovine heart)                 | 13.4                | 0.728  | 1.71                                      | 1.190                     |
| Myoglobin (horse heart)                     | 16.9                | 0.741  | 2.04                                      | 1.105                     |
| α-Chymotrypsin (bovine pancreas)            | 21.6                | 0.736  | 2.40                                      | 1.130                     |
| Crototoxin (rattlesnake)                    | 29.9                | 0.704  | 3.14                                      | 1.221                     |
| Concanavalin B (jack bean)                  | 42.5                | 0.730  | 3.50                                      | 1.247                     |
| Diphtheria toxin                            | 70.4                | 0.736  | 4.60                                      | 1.296                     |
| Cytochrome oxidase ( <i>P. aeruginosa</i> ) | 89.8                | 0.730  | 5.80                                      | 1.240                     |
| Lactate dehydrogenase H (chicken)           | 150                 | 0.740  | 7.31                                      | 1.330                     |
| Catalase (horse liver)                      | 222                 | 0.715  | 11.20                                     | 1.246                     |
| Fibrinogen (human)                          | 340                 | 0.725  | 7.63                                      | 2.336                     |
| Hemocyanin (squid)                          | 612                 | 0.724  | 19.50                                     | 1.358                     |
| Glutamate dehydrogenase (bovine liver)      | 1015                | 0.750  | 26.60                                     | 1.250                     |
| Turnip yellow mosaic virus protein          | 3013                | 0.740  | 48.80                                     | 1.470                     |

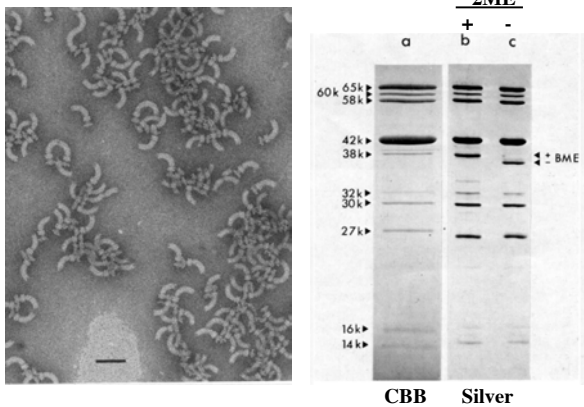
Source: Smith, M.H., in Sober, H.A. (Ed.), *Handbook of Biochemistry and Molecular Biology* (2nd ed.), p. C-10, CRC Press (1970).

Figure 6-30 Zonal ultracentrifugation.

- (1) ゾーン超遠心分離法 (シヨ糖密度勾配)
- (2) 平衡密度勾配超遠心分離 (CsCl密度勾配)



EM and SDS gels of HBB preparations



等電点電気泳動：小分子量 (300~600D) のオリゴマーで等電点の連続的に異なるものを作り (キャリアーアンフォライト)、電圧をかける。尿素を加えることが多い。

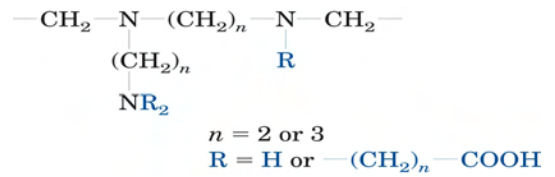
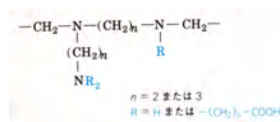


Figure 6-26 General formula of the ampholytes used in isoelectric focusing.

2次元電気泳動 (O'Farrellの電気泳動)



アンホライト (両性電解質)

大腸菌を<sup>14</sup>Cアミノ酸でラベルし、電気泳動後、オートラジオグラフィで検出

<sup>35</sup>S-labeled HBB preparation

