

# Toward a Whole-Cell Model of *Mycoplasma genitalium*

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“The **ultimate test** of understanding a simple cell, more than being able to build one, would be to **build a computer model of the cell**, because that really requires understanding at a deeper level.”

Clyde Hutchison  
The New York Times, 1999

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With a whole cell model, we could...

- Interpret large-scale datasets
- Predict complex behaviors
- Design novel organisms rationally
- Reduce development time and cost

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*E. coli* (FBA): 600 metabolic genes



Expanded “minimal cell” model



*M. pneumoniae* “tour de force”

1999

2001

2003

2005

2007

2009



E-cell: 127 genes



BioCyc: 160 genomes



*H. salinarium* regulatory model

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Question 1: Can we build a whole-cell model today?

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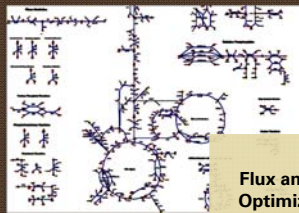
Lessons of the past decade

Whole-cell modeling requires a *variety* of approaches

Integration of diverse approaches will be essential

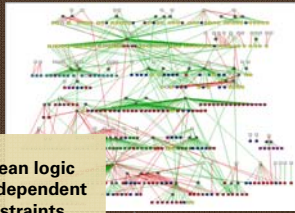
6

Carbon/Energy Metabolism



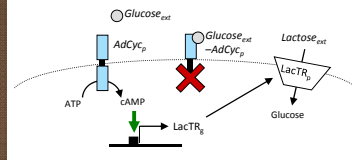
Flux analysis  
Optimization

Transcriptional Regulation



Boolean logic  
Time-dependent constraints

ODEs  
Detailed kinetics



Signal Transduction

JTB 2001  
JTB 2003  
Nature 2004  
Bioinformatics 2008

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Transport

Metabolism

Gene Expression

Cell Division

Modules

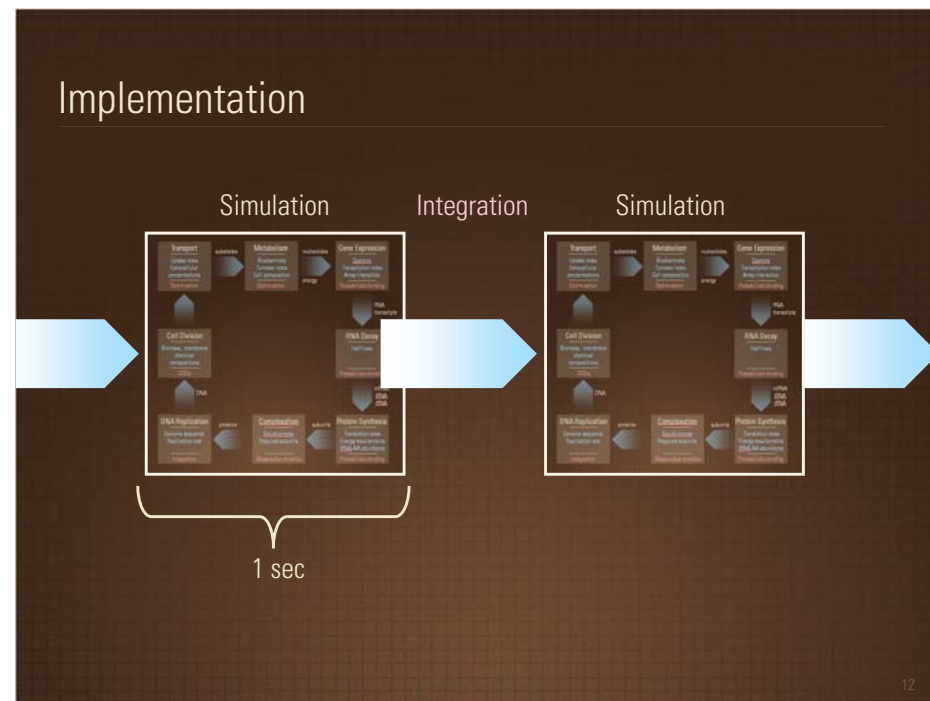
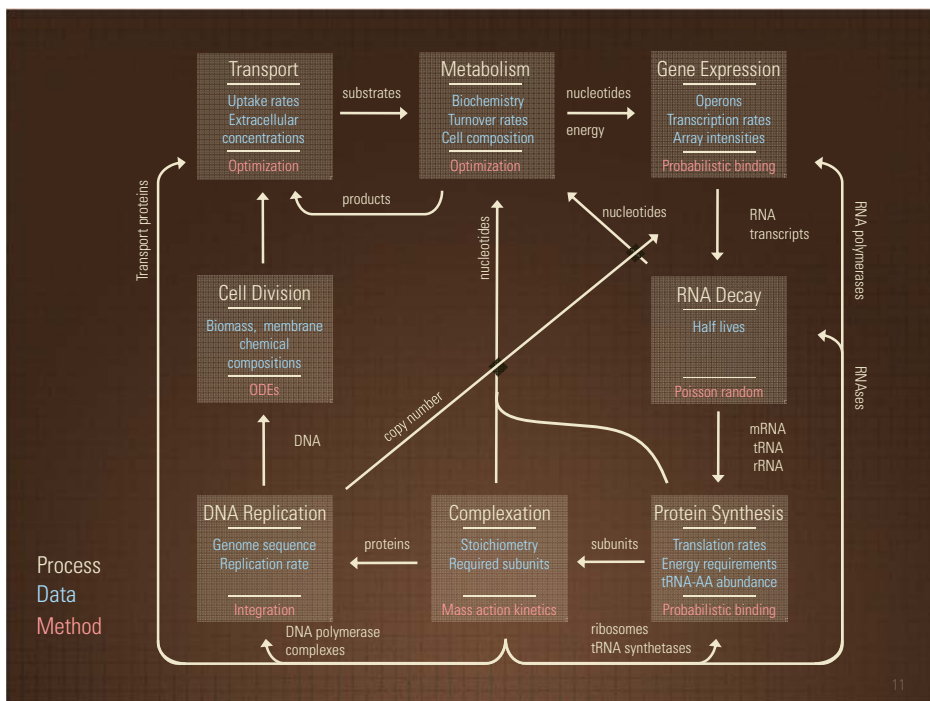
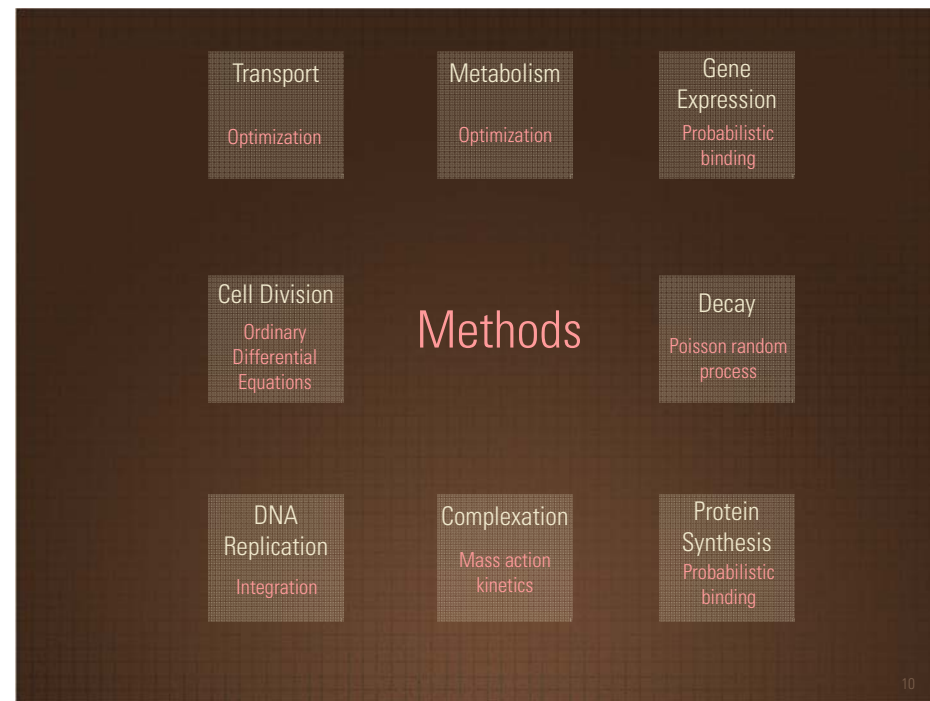
Decay

DNA Replication

Complexation

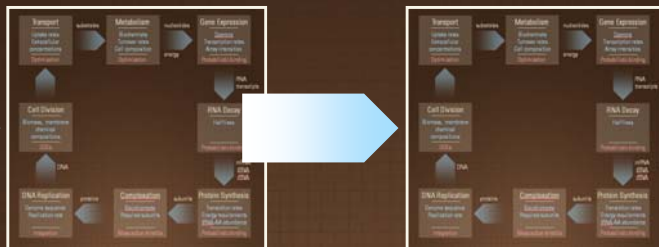
Protein Synthesis

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# Implementation

Simulation      Integration      Simulation



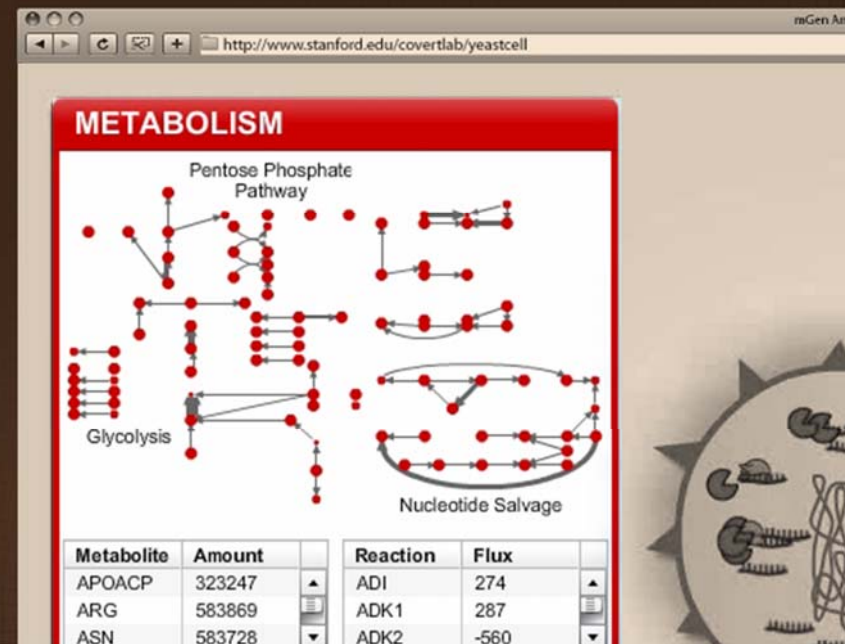
Metabolite concentrations  
Protein monomers  
Complexes

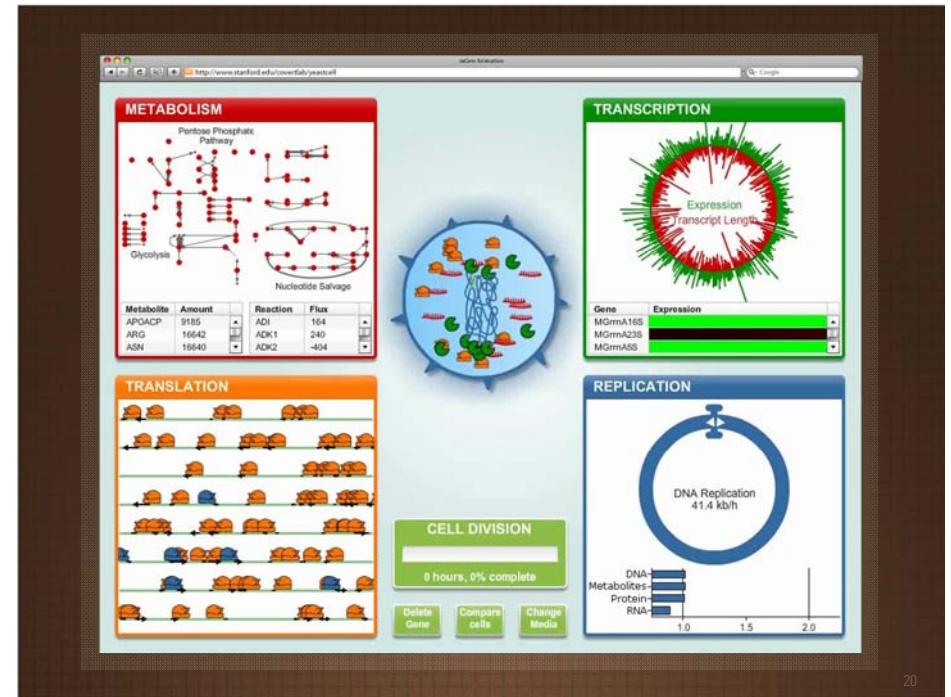
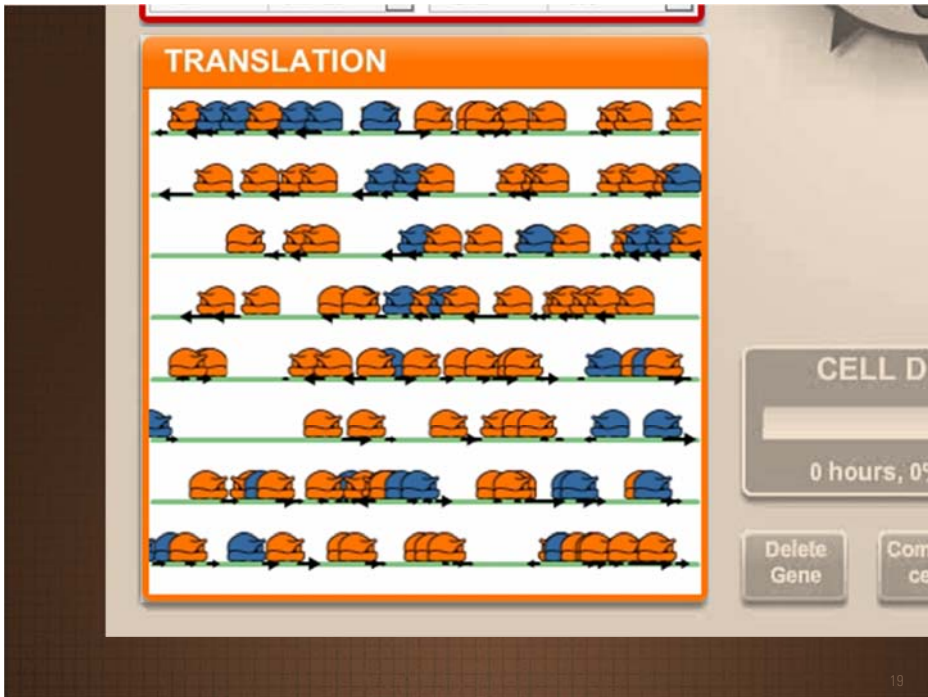
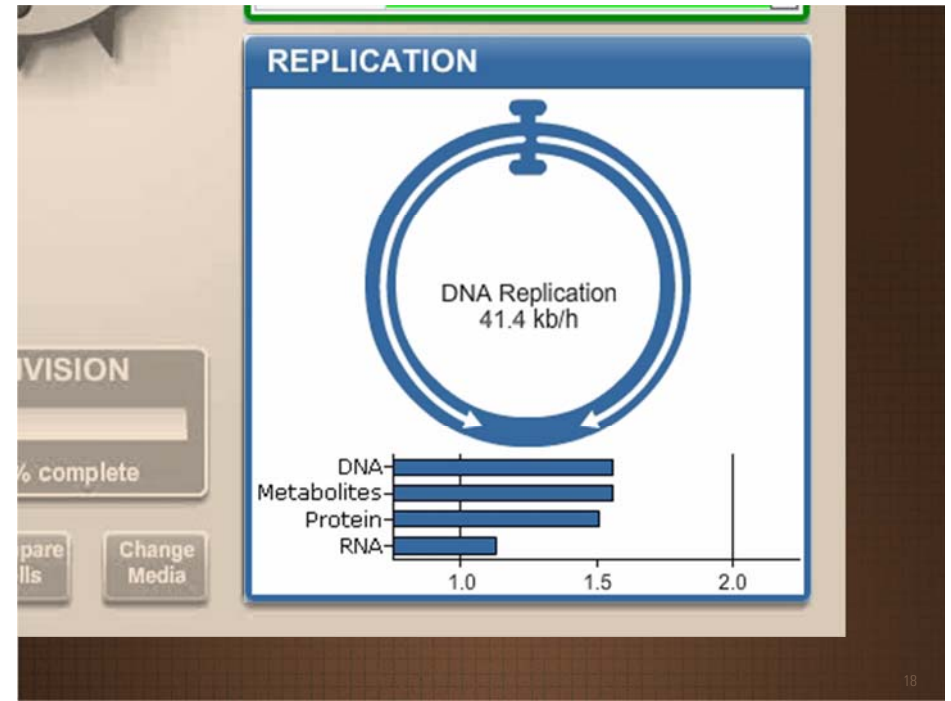
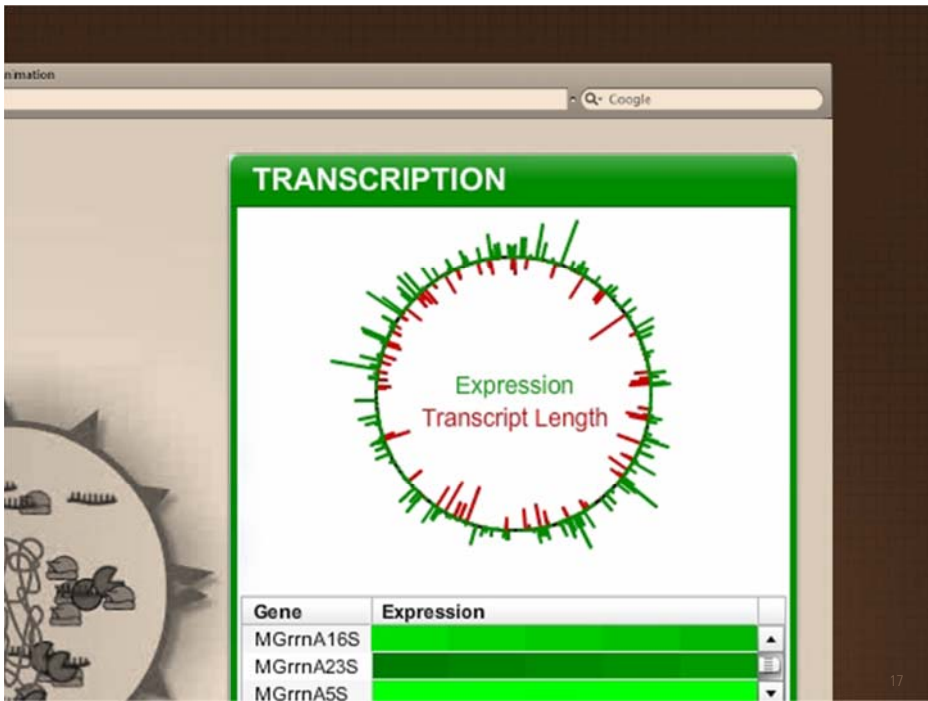
Polymerase positions  
Ribosome positions  
RNAs

Metabolic fluxes  
Cell mass  
...

# *Mycoplasma genitalium*

Small number of genes  
Annotated genome sequence  
Homology to model bacteria  
Free-living for culture



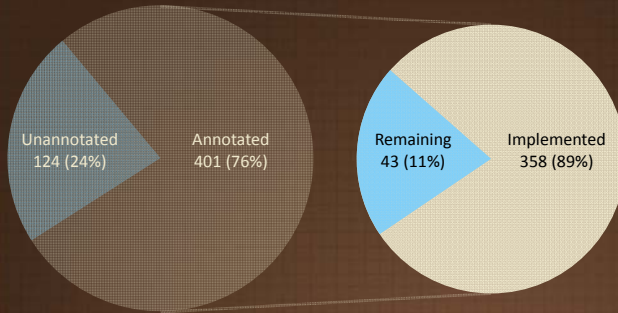


# Project status (8/2010)

*M. genitalium* : 525 genes

Model goal : 401 genes

- Metabolism 147
- Translation 70
- tRNA aminoacylation 60
- RNA modification 13
- DNA replication 12
- Protein decay 9
- Protein translocation 9
- Attachment 9
- Transcription 8
- Protein folding 6
- Ribosome assembly 4
- Protein processing 4
- Protein modification 3
- RNA decay 2
- DNA damage/repair 18
- Cytokinesis 8
- RNA processing 6
- Transcription regulation 6
- DNA supercoiling 5



# Question 2: Can we predict the outcome of perturbations?

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  |     | 18  | 19  | 20  | 21  | 22  | 23  |     |
| 24  | 25  | 26  | 27  | 28  | 29  | 30  | 31  | 32  | 33  | 34  | 35  | 36  | 37  | 38  | 39  | 40  | 41  | 42  | 43  | 44  | 45  | 46  |
| 47  | 48  | 49  | 50  | 51  | 52  | 53  | 54  | 55  | 56  | 57  | 58  | 59  | 60  | 61  | 62  | 63  | 64  | 65  | 66  | 67  | 68  | 69  |
| 70  | 71  | 72  | 73  | 74  | 75  | 76  | 77  | 78  | 79  | 80  | 81  | 82  | 83  | 84  | 85  | 86  | 87  | 88  | 89  | 90  | 91  | 92  |
| 93  | 94  | 95  | 96  | 97  | 98  | 99  | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 |
| 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 136 | 137 | 138 |
| 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 |
| 162 | 163 | 164 | 165 | 166 | 167 | 168 | 169 | 170 | 171 | 172 | 173 | 174 | 175 | 176 | 177 | 178 | 179 | 180 | 181 | 182 | 183 | 184 |
| 185 | 186 | 187 | 188 | 189 | 190 | 191 | 192 |     | 194 | 195 | 196 | 197 | 198 | 199 | 200 | 201 | 202 | 203 | 204 | 205 | 206 | 207 |
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| 231 | 232 | 233 | 234 | 235 | 236 | 237 | 238 | 239 | 240 | 241 | 242 | 243 | 244 | 245 | 246 | 247 | 248 | 249 | 250 | 251 | 252 | 253 |
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| 277 | 278 | 279 | 280 | 281 | 282 | 283 | 284 | 285 | 286 | 287 | 288 | 289 | 290 | 291 | 292 | 293 | 294 | 295 | 296 | 297 | 298 | 299 |
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| 392 | 393 | 394 | 395 | 396 | 397 | 398 | 399 | 400 | 401 | 402 | 403 | 404 | 405 | 406 | 407 | 408 | 409 | 410 | 411 | 412 | 414 | 414 |
|     | 417 | 418 | 419 | 420 | 421 | 422 | 423 | 424 | 425 | 426 | 427 | 428 | 429 | 430 | 431 | 432 | 433 | 434 | 435 | 437 |     |     |
| 438 | 439 | 440 | 441 | 442 | 443 | 444 | 445 | 446 | 447 | 448 | 449 | 450 | 451 | 452 | 453 | 454 | 455 | 456 | 457 | 458 | 459 | 460 |
| 461 | 462 | 463 | 464 | 465 | 466 | 467 | 468 | 469 | 470 | 471 | 472 | 473 | 474 | 475 | 476 | 477 | 478 | 479 | 480 | 481 | 482 | 483 |
| 484 | 485 | 486 | 487 | 488 | 489 | 490 | 491 | 492 | 493 | 494 | 495 | 496 | 497 | 498 | 499 | 500 | 501 | 502 | 503 | 504 | 505 | 506 |
| 507 | 508 | 509 | 510 | 511 | 512 | 513 | 514 | 515 | 516 | 517 | 518 | 519 | 520 | 521 | 522 | 523 | 524 | 525 | 526 | 527 | 528 | 529 |

■ Correctly predicted (230)  
■ Incorrectly predicted (19)

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
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| 93  | 94  | 95  | 96  | 97  | 98  | 99  | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 |
| 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 136 | 137 | 138 |
| 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 |
| 162 | 163 | 164 | 165 | 166 | 167 | 168 | 169 | 170 | 171 | 172 | 173 | 174 | 175 | 176 | 177 | 178 | 179 | 180 | 181 | 182 | 183 | 184 |
| 185 | 186 | 187 | 188 | 189 | 190 | 191 | 192 |     | 194 | 195 | 196 | 197 | 198 | 199 | 200 | 201 | 202 | 203 | 204 | 205 | 206 | 207 |
| 208 | 209 | 210 | 211 | 212 | 213 | 214 | 215 | 216 | 217 | 218 | 219 | 220 | 221 | 222 | 223 | 224 | 225 | 226 | 227 | 228 | 229 | 230 |
| 231 | 232 | 233 | 234 | 235 | 236 | 237 | 238 | 239 | 240 | 241 | 242 | 243 | 244 | 245 | 246 | 247 | 248 | 249 | 250 | 251 | 252 | 253 |
| 254 | 255 | 256 | 257 | 258 | 259 | 260 | 261 | 262 | 263 | 264 | 265 | 266 | 267 | 268 | 269 | 270 | 271 | 272 | 273 | 274 | 275 | 276 |
| 277 | 278 | 279 | 280 | 281 | 282 | 283 | 284 | 285 | 286 | 287 | 288 | 289 | 290 | 291 | 292 | 293 | 294 | 295 | 296 | 297 | 298 | 299 |
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| 323 | 324 | 325 | 326 | 327 | 328 | 329 | 330 | 331 | 332 | 333 | 334 | 335 | 336 | 337 | 338 | 339 | 340 | 341 | 342 | 343 | 344 | 345 |
| 346 | 347 | 348 | 349 | 350 | 351 | 352 | 353 | 354 | 355 | 356 | 357 | 358 | 359 | 360 | 361 | 362 | 363 | 364 | 365 | 366 | 367 | 368 |
| 369 | 370 | 371 | 372 | 373 | 374 | 375 | 376 | 377 | 378 | 379 | 380 | 381 | 382 | 383 | 384 | 385 | 386 | 387 | 388 | 389 | 390 | 391 |
| 392 | 393 | 394 | 395 | 396 | 397 | 398 | 399 | 400 | 401 | 402 | 403 | 404 | 405 | 406 | 407 | 408 | 409 | 410 | 411 | 412 | 414 | 414 |
|     | 417 | 418 | 419 | 420 | 421 | 422 | 423 | 424 | 425 | 426 | 427 | 428 | 429 | 430 | 431 | 432 | 433 | 434 | 435 | 437 |     |     |
| 438 | 439 | 440 | 441 | 442 | 443 | 444 | 445 | 446 | 447 | 448 | 449 | 450 | 451 | 452 | 453 | 454 | 455 | 456 | 457 | 458 | 459 | 460 |
| 461 | 462 | 463 | 464 | 465 | 466 | 467 | 468 | 469 | 470 | 471 | 472 | 473 | 474 | 475 | 476 | 477 | 478 | 479 | 480 | 481 | 482 | 483 |
| 484 | 485 | 486 | 487 | 488 | 489 | 490 | 491 | 492 | 493 | 494 | 495 | 496 | 497 | 498 | 499 | 500 | 501 | 502 | 503 | 504 | 505 | 506 |
| 507 | 508 | 509 | 510 | 511 | 512 | 513 | 514 | 515 | 516 | 517 | 518 | 519 | 520 | 521 | 522 | 523 | 524 | 525 | 526 | 527 | 528 | 529 |

■ Correctly predicted (230)  
■ Incorrectly predicted (19)

92.4% of KO simulations qualitatively correct

False Essential

- MG\_010 DNA primase-related protein
- MG\_039 FAD-dependent glycerol-3-phosphate dehydrogenase
- MG\_066 Transketolase
- MG\_112 Ribulose-phosphate 3-epimerase
- MG\_271 Dihydroliipoamide dehydrogenase
- MG\_298 Chromosome segregation protein
- MG\_315 DNA polymerase III, delta subunit, putative
- MG\_398 ATP synthase F1, epsilon subunit

False Non-essential

- MG\_030 Uracil phosphoribosyltransferase
- MG\_038 Glycerol kinase
- MG\_049 Purine nucleoside phosphorylase
- MG\_050 Deoxyribose-phosphate aldolase
- MG\_119 ABC transporter subunit (ribose?)
- MG\_120 ABC transporter subunit (ribose?)
- MG\_259 Modification methylase, HemK family
- MG\_323 Potassium uptake protein, TrkA family
- MG\_345 isoleucyl-tRNA synthetase
- MG\_369 DAK2 phosphatase domain protein
- MG\_372 Thiamine biosynthesis/tRNA modification protein ThiI

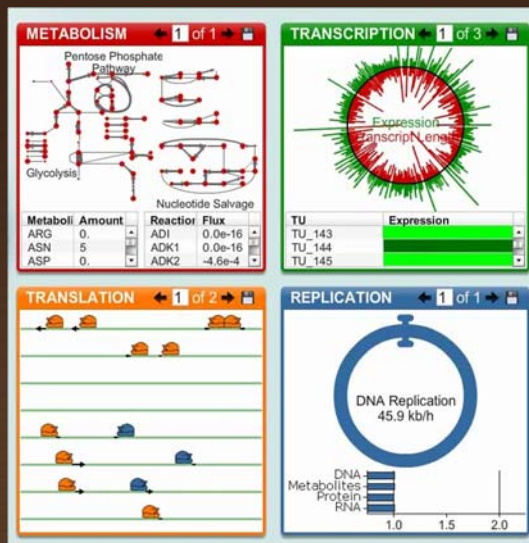
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## MG\_471 knockout simulation



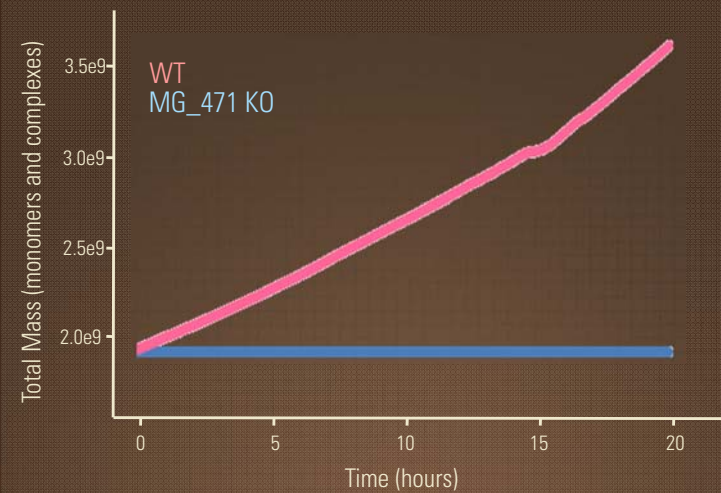
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## MG\_471 knockout simulation



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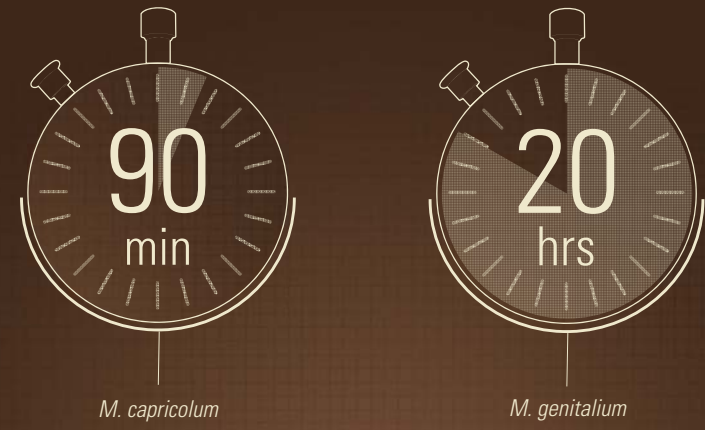
Total protein production in two single-cell *M. genitalium* simulations



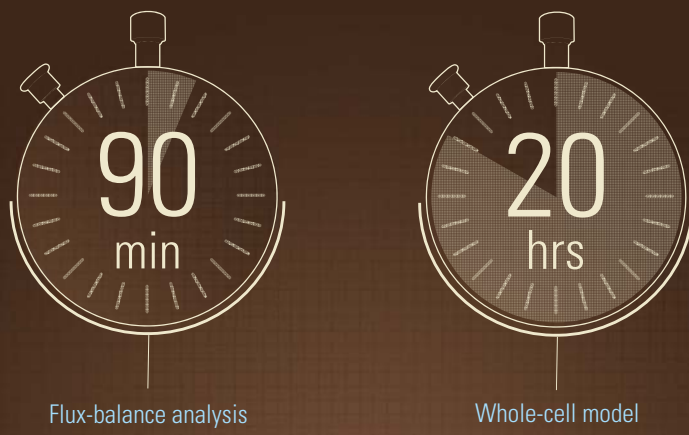
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### Question 3: Can we interpret complex phenotypes?

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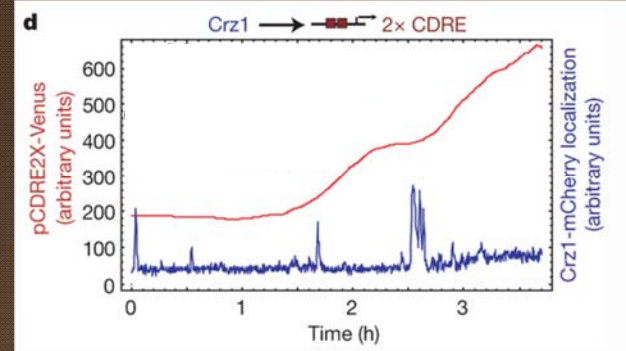


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### Crz1 localization and Reporter expression in a single yeast cell

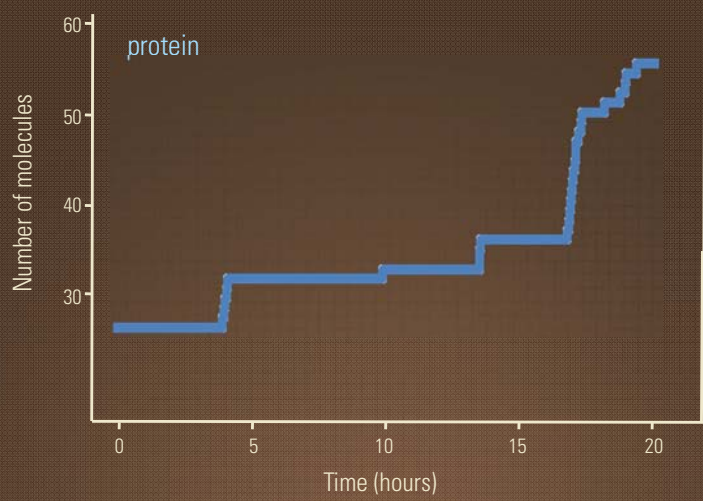


Cai et al., *Nature* 2008

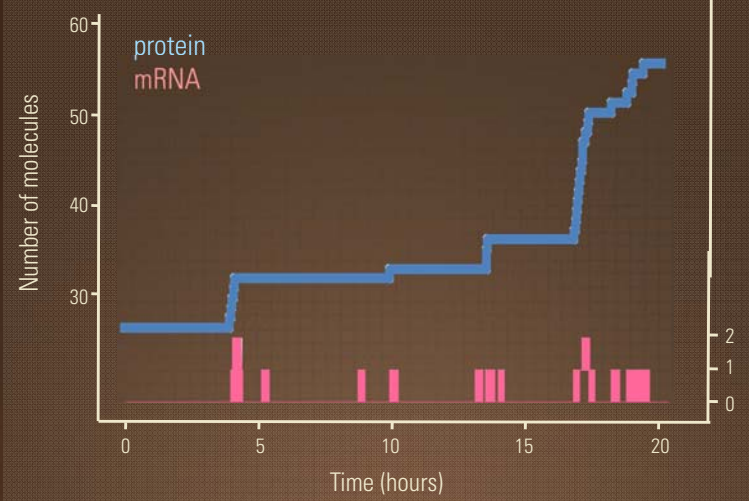
32



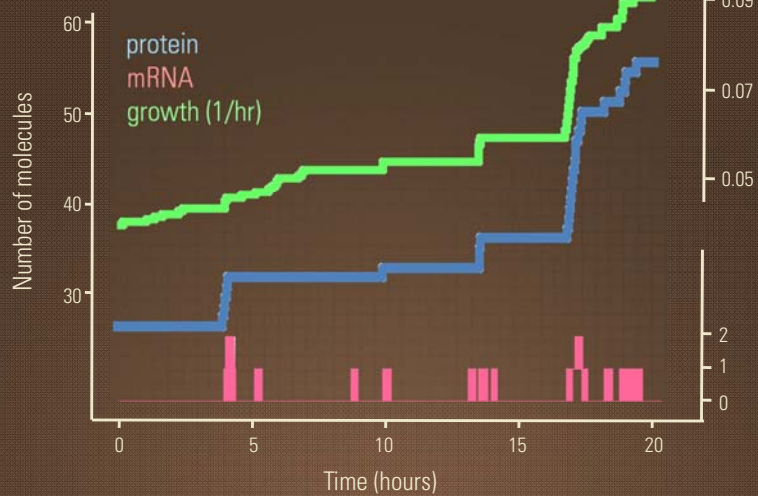
FoLD expression in a single-cell *M. genitalium* simulation



FoLD expression in a single-cell *M. genitalium* simulation



FoLD expression in a single-cell *M. genitalium* simulation



### ATP Synthase

MG\_398: *atpC*  
 MG\_399: *atpD*  
 MG\_400: *atpG*  
 MG\_401: *atpA*  
 MG\_402: *atpH*  
 MG\_403: *atpF*  
 MG\_404: *atpE*  
 MG\_405: *atpB*

### Pyruvate Dehydrogenase

MG\_274: *pdhA*  
 MG\_272: *pdhC*  
  
 Methylene THF Enzyme  
 MG\_013: *folD*

### tRNAs

MG\_483: tRNA/CYS  
 MG\_496: tRNA/TRP  
 MG\_504: tRNA/TRP

### Riboflavin Kinase

MG\_145: *ribF*

Can we **build** a whole cell model today?

Can we **predict** the outcome of perturbations?

Can we **interpret** complex phenotypes?

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## Acknowledgements

### Covert Lab

Whole-cell modeling

Jonathan Karr  
Jayodita Sanghvi

NF- $\kappa$ B single cell dynamics

Timothy Lee  
Jake Hughey

Virus-pathogen interactions

Nate Maynard  
Elsa Birch

### Support

NIH Director's Pioneer Award  
NIH Pathway to Independence Award



[covertlab.stanford.edu](http://covertlab.stanford.edu)

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