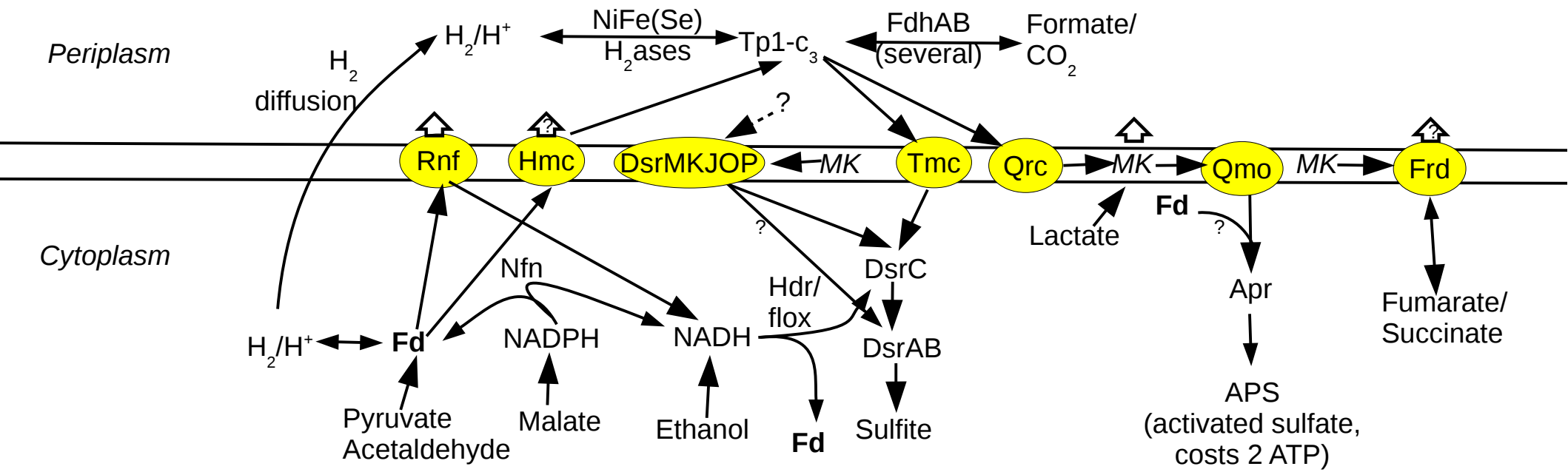
Proposed Scenarios for Electron Flow During Sulfate Reduction By *Desulfovibrio alaskensis* G20



For these scenarios, we make *simplifying and uncertain* assumptions about Qmo and DsrMKJOP:

2 electrons to reduce APS to sulfite:

- 1 from Fd
- 1 from MK

6 electrons to reduce sulfite to sulfide:

- 2 from DsrC (which can be from MK via DsrMKJOP)
- 4 from MK via DsrMKJOP

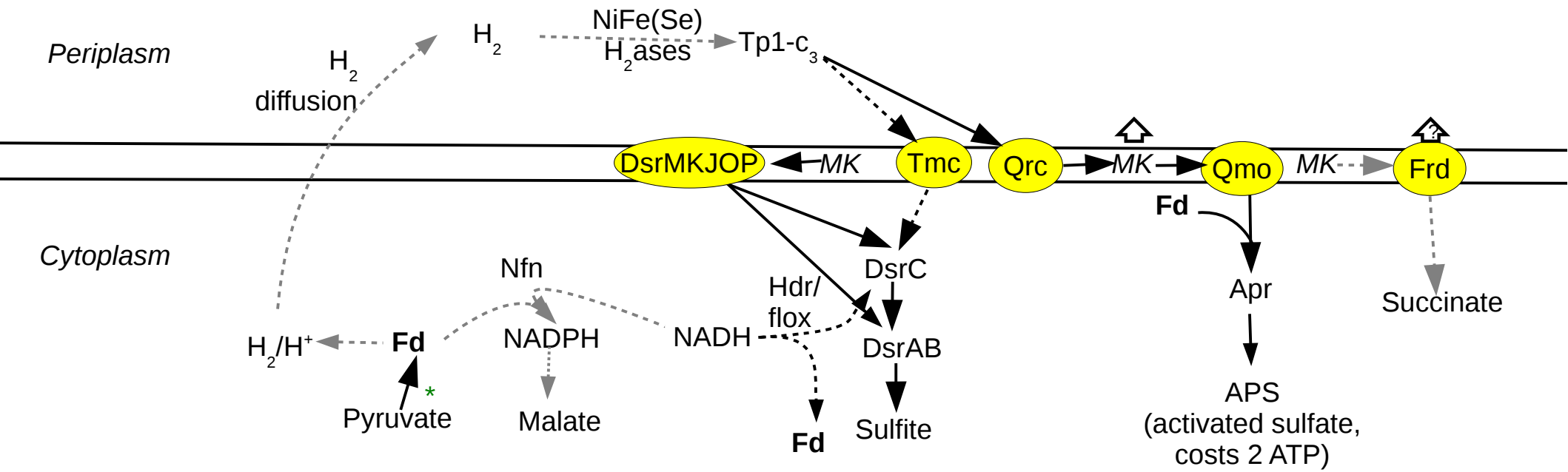
Overall, to reduce 1 molecule of APS, need reduced forms of

- 0.5 Fd + 1 DsrC + 2.5 MK

A recurring issue with this model is: where does all the reduced MK for sulfite reduction come from?

- Alternatively, can all 6 electrons for sulfite reduction come from DsrC, perhaps by reversibility of DsrMKJOP?

Scenario for Pyruvate/Sulfate



Per sulfate reduced:

- 4 pyruvate → 4 acetyl-CoA + 4 reduced Fd
- 2 net ATP from substrate-level phosphorylation

Pyruvate can also be reduced to malate, converted to fumarate, and further reduced to succinate

- This requires NADPH, which might be formed by Nfn

To reduce 1 APS, need: 2.5 reduced MK, 1 reduced DsrC, 0.5 reduced Fd

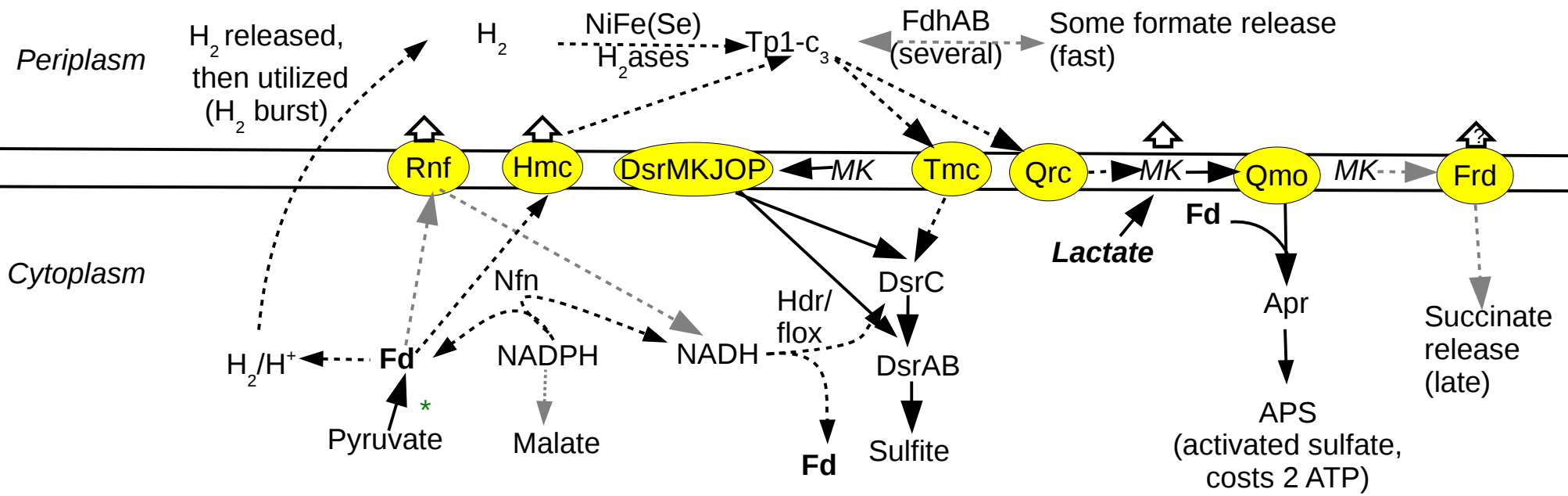
- So need to convert 3.5 Fd → 1 DsrC + 2.5 MK

DsrC is reduced by Hdr/flox (which requires NADH), Tmc (which requires reduced Tp1-c_3), or DsrMKJOP (from reduced MK)

- NADH is reduced by Rnf (not shown) or by Hdr/flox (in reverse)?
- Tp1-c_3 is reduced by hydrogen cycling, formate cycling (not shown), or possibly Hmc (not shown)

MK is reduced by Qrc, which requires reduced Tp1-c_3

Scenario for Lactate/Sulfate



Per sulfate reduced:

2 lactate → 2 pyruvate + 2 reduced MK

2 pyruvate → 2 acetyl-CoA + 2 reduced Fd

0 net ATP from substrate-level phosphorylation

To reduce 1 APS, need: 2.5 reduced MK, 1 reduced DsrC, 0.5 reduced Fd

So need to convert 1.5 Fd → 1 DsrC + 0.5 MK

0.5 MK from $Tp1c_3$ and Qrc, with $Tp1c_3$ reduced by Fd via H_2 cycling or Hmc

Or, if >2 lactate oxidized per sulfate reduced, and Fd is converted to H_2, then excess MK is achieved, and net ATP is increased because not all is used for activating APS

1 DsrC is reduced by Tmc (from reduced $Tp1c_3$) or DsrMKJOP (via Qrc and MK)

Some pyruvate is reduced to malate, which is converted to fumarate, and reduced to succinate

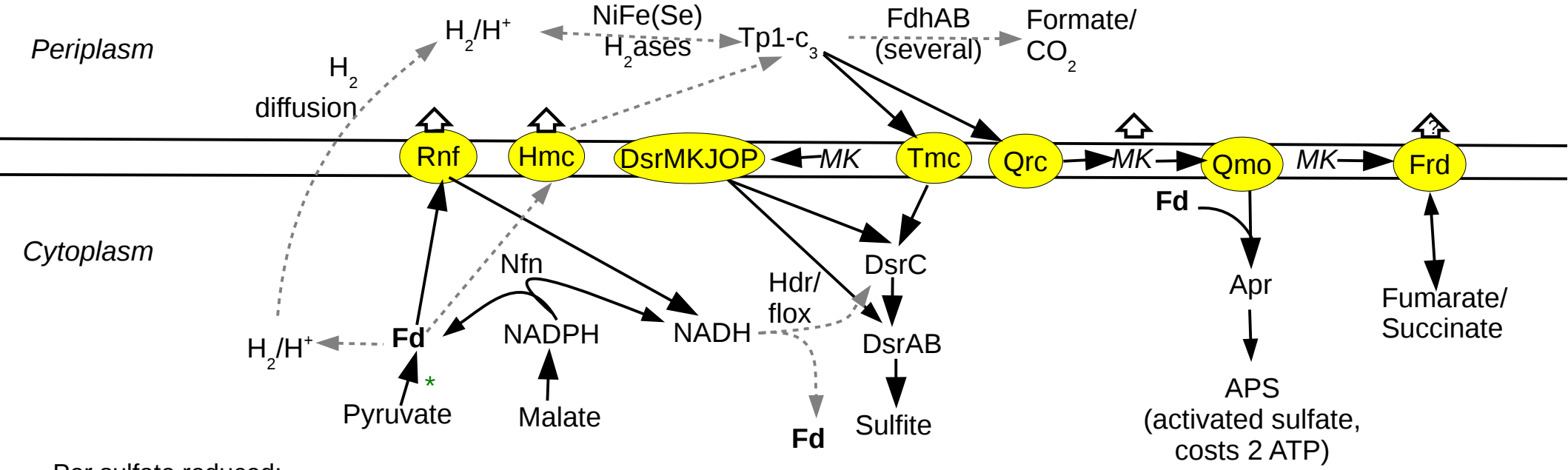
The source of NADPH is unclear (Nfn & Rnf?), as is the source of excess MK

Energy conservation by Qmo and by H_2 release, H_2 cycling, Hmc, or Rnf with Hdr/flox (lots of redundancy)

Scenario for Fumarate/Sulfate

Important for fitness
Sometimes important
Not important, but carries flux (end products detected)

Overview:



Per sulfate reduced:

2 fumarate ↔ 2 malate → 2 pyruvate + 2 reduced NADPH
2 pyruvate → 2 acetyl-CoA + 2 reduced Fd
+0 net ATP from substrate-level phosphorylation

Fumarate can also be reduced to succinate, but we found no net succinate reduction:
succinate appeared early and then disappeared. Nevertheless, Frd was important for fitness.

To reduce 1 APS, need 2.5 reduced MK, 1 reduced DsrC, 0.5 reduced Fd

Nfn converts NADPH to reduced NADH + reduced Fd

Neither Hdr/flox cluster was important for fitness, but it is expected to consume the NADH from Nfn (and Rnf)
And it would produce DsrC. (Are hdr/flox-1,-2 redundant? Is there another path from NADH?)

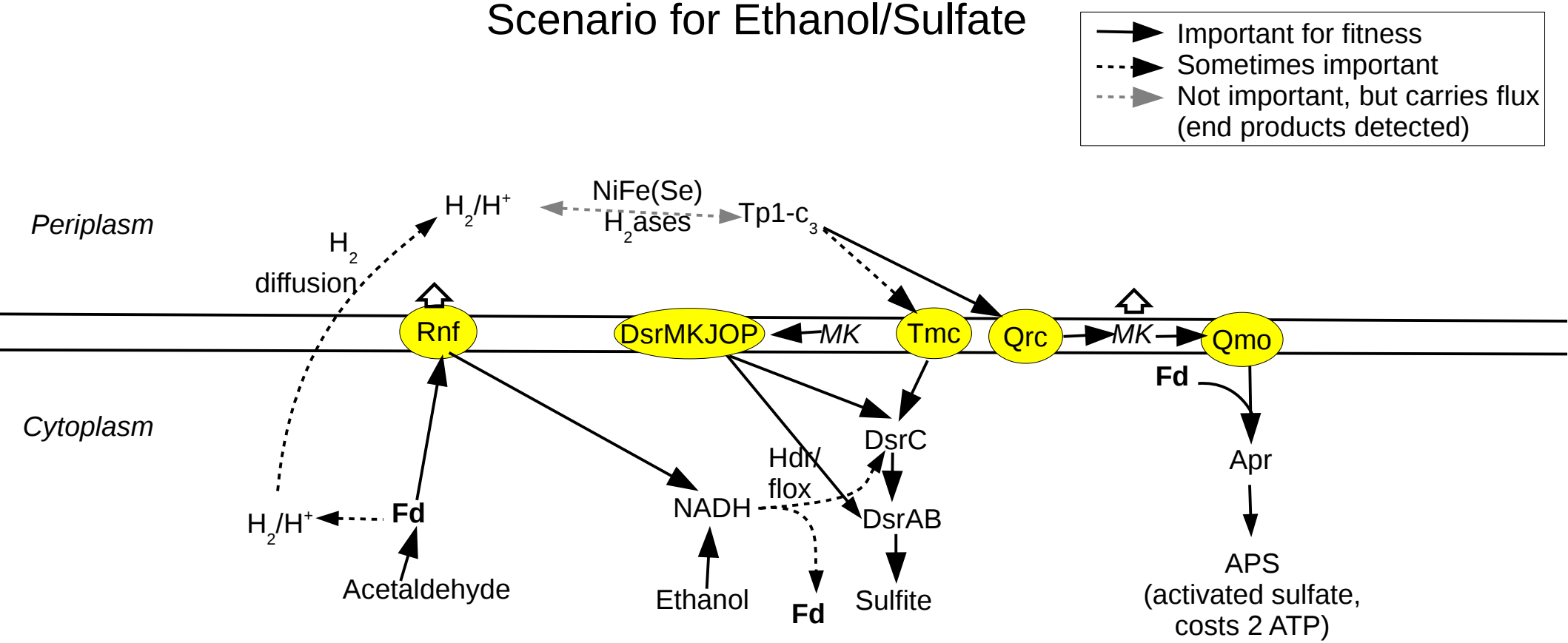
The path to Tp1-c_3 is unclear but could involve hydrogen cycling or possibly Hmc (mean fitness = -0.1)
(We did not measure H_2 release but assume that it occurs.)

Given reduced Tp1-c_3, Tmc could reduce DsrC, and Qrc could reduce MK

Energy conservation by Nfn, Rnf, Qrc/Qmo, and possibly hydrogen cycling or Hmc

Note: electron flow on malate/sulfate is probably very similar to this.

Scenario for Ethanol/Sulfate



Per sulfate reduced:

2 ethanol → 2 acetaldehyde + 2 reduced NADH

2 acetaldehyde → 2 acetate + 2 reduced Fd

No substrate-level phosphorylation

To reduce 1 APS, need 2.5 reduced MK, 1 reduced DsrC, 0.5 reduced Fd

So need to convert 2 NADH + 1.5 Fd → 1 DsrC + 2.5 MK

Rnf together with Hdr/flox convert reduced Fd or NADH to reduced DsrC, with ion pumping

Qrc produces reduced MK, but how do electrons reach Tp1-c_3?

H_2 cycling?

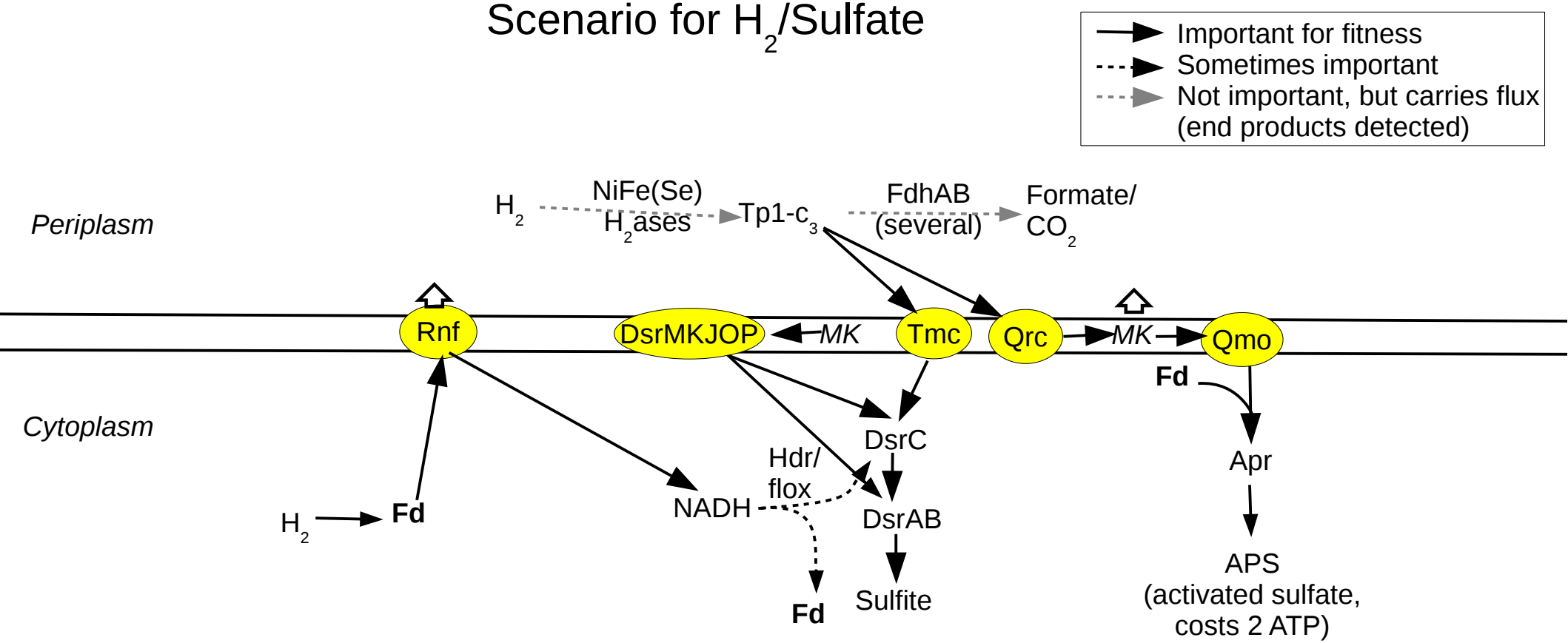
DsrMKJOP could be an alternate source of reduced MK?

Hmc? (but Hmc is detrimental to fitness)

Or, most of the electrons for sulfite reduction come from DsrC and not from MK?

Note: electron flow/sulfate on choline is expected to be similar, as choline is disproportionated to ethanol + acetyl-phosphate, but this allows for substrate-level phosphorylation (0 net ATP per sulfate reduced)

Scenario for H_2/Sulfate



Per sulfate reduced:

4 H2 → 8 H^+ + 4 reduced Tp1-c_3, and/or

4 H2 → 8 H^+ + 4 reduced Fd

No substrate-level phosphorylation

To reduce 1 APS need: 2.5 reduced MK, 1 reduced DsrC, 0.5 reduced Fd

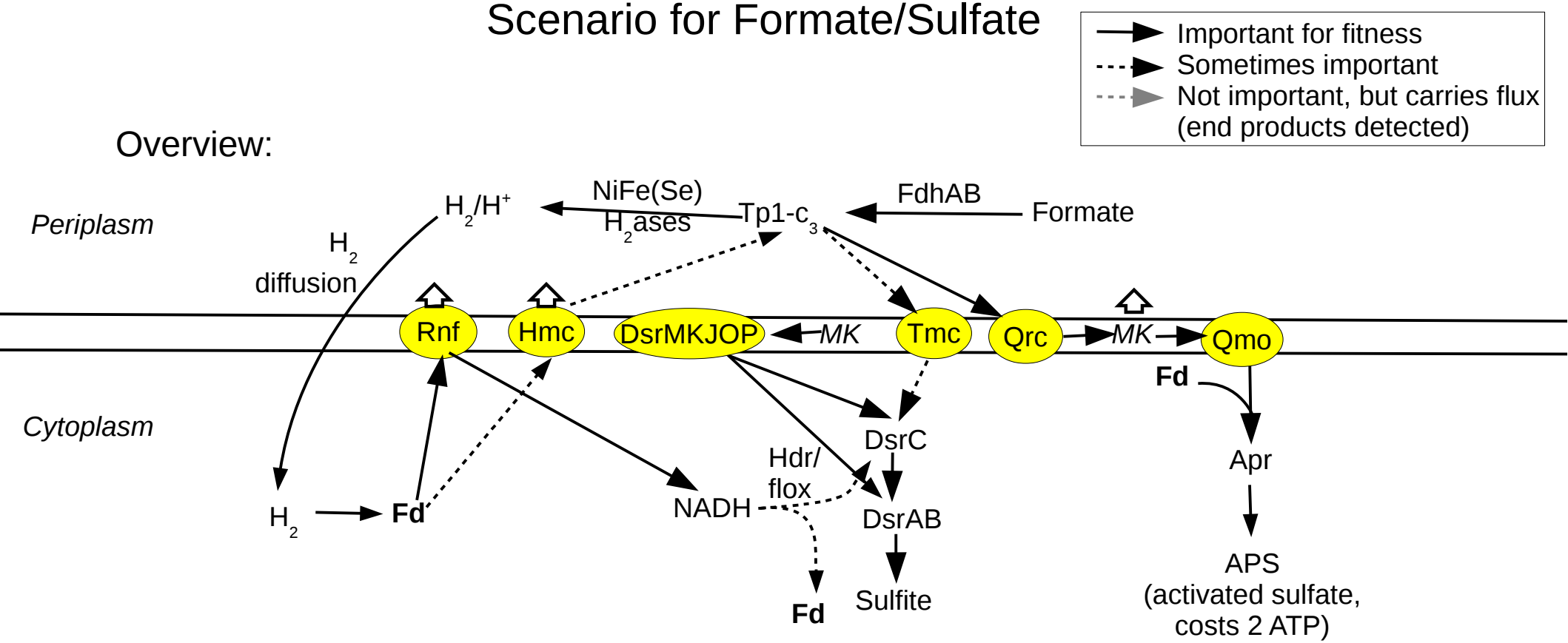
Rnf together with Hdr/flox convert reduced Fd to reduced DsrC, with ion pumping

Qrc reduces MK (from Tp1-c_3)

Tmc reduces DsrC from Tp1-c_3)

Energy conservation by Rnf, Hdr/flox, and Qrc/Qmo

Scenario for Formate/Sulfate



Per sulfate reduced:

4 formate → 4 CO_2 + 4 reduced $Tp1c_3$

No substrate-level phosphorylation

To reduce 1 APS, need 2.5 reduced MK, 1 reduced DsrC, 0.5 reduced Fd

Hmc can reduce Tp1-c_3 if oxidation is primarily cytoplasmic (due to cross-feeding of H_2)?

Qrc reduces MK

Conversion to H_2 and diffusion into cytoplasm allows reduction of Fd by cytoplasmic H_2ase

Rnf together with Hdr/flox can convert reduced Fd to reduced DsrC, with ion pumping

Or, Tmc produces reduced DsrC

Energy conservation by Rnf, Hdr/flox, Qrc/Qmo, and possibly Hmc