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REVIEW

Pseudomonad reverse carbon catabolite repression, interspecies metabolite exchange, and consortial division of labor

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Abstract

Microorganisms acquire energy and nutrients from dynamic environments, where substrates vary in both type and abundance. The regulatory system responsible for prioritizing preferred substrates is known as carbon catabolite repression (CCR). Two broad classes of CCR have been documented in the literature. The best described CCR strategy, referred to here as classic CCR (cCCR), has been experimentally and theoretically studied using model organisms such as *Escherichia coli*. cCCR phenotypes are often used to generalize universal strategies for fitness, sometimes incorrectly. For instance, extremely competitive microorganisms, such as Pseudomonads, which arguably have broader global distributions than *E. coli*, have achieved their success using metabolic strategies that are nearly opposite of cCCR. These organisms utilize a CCR strategy termed 'reverse CCR' (rCCR), because the order of preferred substrates is nearly reverse that of cCCR. rCCR phenotypes prefer organic acids over glucose, may or may not select preferred substrates to optimize growth rates, and do not allocate intracellular resources in a manner that produces an overflow metabolism. cCCR and rCCR have traditionally been interpreted from the perspective of monocultures, even though most microorganisms live in consortia. Here, we review the basic tenets of the two CCR strategies and consider these phenotypes from the perspective of resource acquisition in consortia, a scenario that surely influenced the evolution of cCCR and rCCR. For instance, cCCR and rCCR metabolism are near mirror images of each other; when considered from a consortium basis, the complementary properties of the two strategies can mitigate direct competition for energy and nutrients and instead establish cooperative division of labor.

Keywords Diauxie · Carbon catabolic repression · Reverse carbon catabolic repression · Division of labor · Overflow metabolism

Introduction

Most natural environments are physically, chemically, and temporally complex, with the resident microorganisms exposed to multifactorial selection pressures. It is not possible to optimize all cellular functions simultaneously due to constraints on cellular resources such as anabolic nitrogen or cytoplasmic volume; a concept illustrated by the 'Darwinian Demon' thought exercise [1, 2]. Therefore, microorganisms must dynamically change their phenotypes with environmental fluctuations to maintain fitness [3]. Metabolic regulation of substrate-consumption order is paramount to fitness. There are a few broad strategies that can be utilized when an organism is presented with two potential substrates: (1) utilize the 'optimal' substrate, (2) utilize the less 'optimal' substrate, or (3) co-catabolize both substrates simultaneously (see Box 1 for discussion of 'optimal' substrates). It can be more resource effective to catabolize a single substrate at a time as opposed to expressing multiple catabolic pathways simultaneously [4, 5]; although, exceptions exist [6]. Carbon catabolite repression (CCR) is a global regulation system that controls the sequential catabolism of preferred substrates from a milieu (Box 2). CCR is associated with generalist microorganisms that can utilize multiple substrates and exist in environments, where these substrates are often available at varying abundances

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Box 1: 'Optimal' substrates.

Monod's study of *E. coli* as the model organism has influenced what is generally considered 'optimal behavior' in computational biology. Species expressing cCCR have formed the basis of much of modern systems biology studies and their metabolic regulation is the basis of textbooks [26]. Glucose is often considered the optimal substrate preferred by chemoheterotrophs, but the notion of an optimal substrate is worthy of further discussion because no substrate is universally preferred by all microorganisms. The monosaccharide has competitive properties including the flexible production of cellular energy from fermentation, respiration or combinations of the two. Glucose integrates, with nominal enzymatic steps, into central metabolism pathways including the Embden-Meyerhof-Parnas (EMP), Entner-Doudoroff (ED) and pentose-phosphate (PP) pathways. Glucose provides convenient ratios of reducing equivalents to metabolic intermediates necessary for biosynthetic fluxes. However, oligomers like cellobiose and lactose are preferred to glucose by some fermenting anaerobes. Fermenters are often considered cellular energy poor and the transport cost of substrate can be a substantial energy drain [27]. The disaccharides are more energetic on a per molecule basis than glucose, so using an ABC transporter to import a disaccharide has a better energetic payback than using an ABC transporter to import glucose [28, 29]. This strategy comes with a tradeoff, as the cell requires investment into additional enzymes to hydrolyze, phosphorylate, and in the case of lactose, process the intermediates so they can enter glycolysis. rCCR organisms like *P. putida and P. aeruginosa* prefer citrate, succinate, lactate, and acetate over glucose even though these substrates contain less energy per molecule and are not generally fermentable [9]. The necessary respiratory metabolism requires large investments into tricarboxylic acid (TCA) cycle and electron transport chain enzymes as well as a supply of terminal electron acceptor like O_2. Citrate and succinate are more oxidized than glucose which would reduce the electron flux per carbon mole of substrate oxidized. The Pseudomonad growth rate on the organic acids is not necessarily faster than on glucose [30].

These non-glucose substrates are certainly 'optimal' substrates if the appropriate ecological criteria are considered. The designation of glucose as the 'optimal' substrate is analogous to outdated arguments describing an overflow metabolism as a 'wasteful metabolism'; in recent years, this metabolic phenotype has been appreciated as a competitive ecological strategy [31, 32].

Preferred substrate	Formula	ΔG combustion (kJ gmol^{-1})*	Degree of reduction	Example microorganism	Strategy
Cellobiose	$C_{12}H_{22}O_{11}$	-5652 (s)	4	*C. thermocellum*	Cost effective transport, fermentable
Lactose	$C_{12}H_{22}O_{11}$	-5652 (s)	4	*B. longum*	Cost effective transport, reciprocal substrate, fermentable
Glucose	$C_6H_{12}O_6$	-2805 (s)	4	*E. coli, B. subtilis*	Cost effective resource investment
Citrate	$C_6H_8O_7$	-1920 (s)	3	*P. putida, P. aeruginosa*	Reciprocal substrate? Division of labor?
Succinate	$C_4H_6O_4$	-1491 (c)	3.5	*P. putida, P. aeruginosa*	Reciprocal substrate? Division of labor?
Lactate	$C_3H_6O_3$	-1368 (l)	4	*P. putida, P. aeruginosa*	Reciprocal substrate? Division of labor?
Acetate	$C_2H_4O_2$	-874 (l)	4	*P. putida, P. aeruginosa*	Reciprocal substrate? Division of labor?

*Combustion values from Bioprocess Engineering Principles, Doran, 2013 ed 2. Academic Press. Combustion value for cellobiose assumed equivalent to lactose.

The cCCR regulation scheme, known under specific conditions as diauxie, has been the focus of much experimental and theoretical research [7–12]. The historical basis of cCCR goes back more than 70 years to Jacques Monod and his observations of *Escherichia coli* growing in the presence of glucose and other sugars [13]. Monod recorded *E. coli* cultures growing exponentially on glucose until depleted, followed by a lag phase, then a second exponential growth phase on the other sugar. Diauxic growth is a trade-off between specializing for a single nutrient and being able to switch between nutrients as conditions change [14]. Committing to one metabolism at a time, necessitates a metabolic shift when the preferred substrate is exhausted, with a resulting temporal delay.

Box 2: Carbon catabolite repression.
Carbon catabolite repression (CCR): A regulatory network which selects 'preferred' carbon sources from a pool of potential carbon sources. The ecological basis of a preferred carbon source is environment dependent and Nature has evolved numerous strategies for optimizing the catabolism of available carbon sources. The best studied and often default example of CCR is that of *E. coli* or *B. subtilus,* which prefer sugars like glucose to other potential carbon sources like organic acids. These model organism have strongly influenced the criteria applied to identify 'optimal' phenotypes in computational biology studies of chemoheterotrophs. However, as science describes a broader range of microbiological physiologies from diverse environments, the number of different CCR strategies is sure to expand. In this review, we consider primarily two CCR strategies defined below.

Classic carbon catabolite repression (cCCR): The textbook example of CCR, first described in model organism *E. coli,* is called cCCR here to distinguish it from the general term CCR and other specific CCR strategies. cCCR phenotypes prefer glucose to other carbon substrates [26].
Reverse carbon catabolite repression (rCCR): A CCR strategy first described in Pseudomonads in 1959 and later termed 'reverse' diauxie or rCCR because the hierarchy of 'preferred' carbon sources was nearly 'reverse' that of cCCR preferences[9,33].

The traditional interpretation of diauxie as being carried out by a single population of organisms, synchronized in function, that sequentially catabolizes preferred and non-preferred substrates has been challenged [15, 16]. Recent research has posited an alternative interpretation; rather than a homogenous population, the observed lag phase can be explained by dynamic shifts in subpopulations possessing different fitness. For instance, during growth on glucose, multiple subpopulations catabolize, the sugar but upon glucose depletion only some subpopulations, in an ecological bet-hedging strategy, are phenotypically prepared to consume the less desirable substrate [17]. The lag in growth between the two sugars corresponds to a shift in subpopulation distributions as fit subpopulations outgrow other subpopulations [17]. Control of these subpopulations has been associated with the regulation of bistable genetic switches [18, 19].

cCCR is not used by all glucose-utilizing microorganisms. Pseudomonads and Acinetobacter [20] utilize substrates in an order almost reverse of cCCR. Therefore, this metabolic regulatory strategy was termed reverse carbon catabolite repression (rCCR) or sometimes reverse diauxie. rCCR utilizing microorganisms are very competitive as evidenced by their broad global distributions and are arguably at least as competitive as the better studied members of the Enterobacter and Firmicutes used to define cCCR. The ecological rationale of the reverse diauxie strategy is still an open question and is discussed in later sections.

The study of cCCR and rCCR has traditionally focused on monocultures for the sake of simplicity, possibly limiting the interpretation of regulatory systems, as most microorganisms have existed on evolutionary timeframes within consortia [21–24]. The organisms have interacted with other species for millennia, and these interactions have influenced access to energy and nutrients [25]. Many genes regulated by the CCR systems such as quorum sensing, biofilm formation, and antibiotic or reactive oxygen species (ROS) tolerance have been studied from the perspective of medical challenges and monocultures, even though these behaviors modulate inter-microbial interactions and strategies for resource acquisition in consortia (see sections below).

This review summarizes (1) cCCR and rCCR molecular mechanisms; (2) theory used to interpret and model CCR phenotypes; and (3) the nexus of CCR, metabolism, and cellular interactions. Collectively, the properties of cCCR and rCCR phenotypes can enhance growth and persistence by enabling cooperation via division of labor, as observed in some medical environments such as chronic wounds, where cCCR and rCCR microorganisms commonly cooccur (Fig. 1).

Molecular mechanisms of CCR

Most CCR studies have been performed in model organisms such as *E. coli* and *Bacillus subtilis,* which prefer glucose over other substrates. The regulatory outcomes of CCR in *E. coli* and *B. subtilis* are similar, though the mechanisms differ (Fig. 2). This review summarizes molecular mechanisms of CCR for context and to highlight similarities and differences between cCCR and rCCR (Table 1). This summary is not exhaustive; there are numerous excellent reviews which discuss finer aspects of CCR molecular biology [7–12].

Molecular mechanisms of cCCR

Gram-negative *E. coli* utilizes two major cCCR regulation mechanisms: (1) inducer exclusion [9, 44, 47, 48] and (2) cyclic AMP (cAMP)-catabolite repressor protein (CRP) regulation of gene protomer activity; although the condition-specific importance of each mechanism is still an

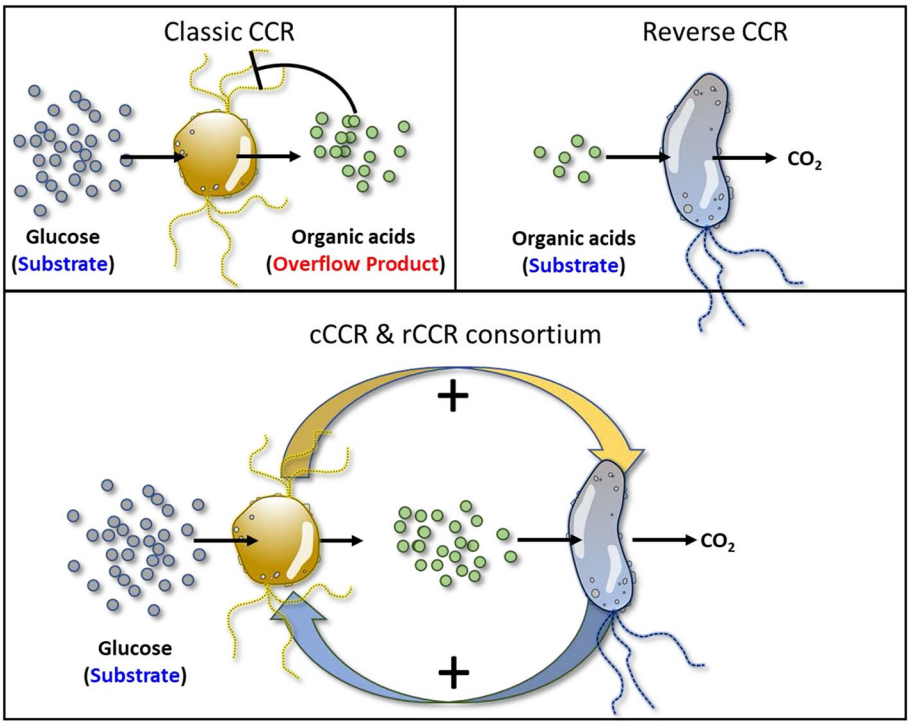

Fig. 1 Illustration of classic carbon catabolite repression (cCCR) phenotype with overflow metabolism and reverse carbon catabolite repression (rCCR) phenotype as well as an illustration of the complementary relationship between the two global metabolism regulators. cCCR microorganisms prefer glucose to other substrates, while rCCR microorganisms prefer organic acids to glucose

open research question [49, 50]. Glucose can enter the cell using the multisubunit phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS). Regulation of cCCR begins with the phosphorylation state of the glucose-specific subunit of the PTS transporter, $EIIA^{Glc}$. $EIIA^{Glc}$ is phosphorylated by PEP via a chain of phosphorylation reactions. In the presence of high glucose flux, the phosphate from $EIIA^{Glc}$ is transferred to the hexose producing glucose-6-phosphate, precluding the phosphate group from activating PTS EIIA components specific for less preferred substrates. This process is known as inducer exclusion, because the excluded, non-preferred substrates are inducers for their own catabolic operons [51]. When glucose flux is low, phosphorylated $EIIA^{Glc}$ accumulates and activates EIIA subunits for less preferred substrates and activates the enzyme adenylate cyclase [35, 52] which catalyzes the production of cAMP [53]. cAMP binds to the catabolite gene activator protein (CAP), forming the cAMP–CAP complex which binds to DNA, activating the promoters of ~ 200 genes for a range of cellular processes [49, 50, 54–57]. The classic example of the CCR inducer exclusion and cAMP-CRP regulation occurs during glucose–lactose diauxic growth [58–61].

CCR is complex, with many levels of modulation in addition to inducer exclusion and cAMP-CRP regulation. Small regulatory RNAs (sRNA) also contribute to post-transcriptional regulation of CCR [10, 62, 63]. sRNA Spot 42 is described as a third pillar of CCR in *E. coli* [10, 64]. Spot 42 represses target genes as opposed to the role of cAMP–CRP, which generally activates gene targets. The Spot 42 regulon has 29 documented genes [65]. Central metabolism intermediates such as a-ketoglutarate, oxaloacetate, PEP, and pyruvate also play important roles in CCR by affecting the levels of cAMP, adding additional complexity to CCR [66, 67].

E. coli cCCR regulation 'glitches' can result in substrate selection that does not enable the fastest growth [68]. *E. coli* cultures growing on a mixture of glucose and lactose and a poor nitrogen source (e.g., arginine, glutamate, and proline) preferentially consume glucose over lactose, but growth on glucose is slower than on lactose due to altered cAMP concentrations. Therefore, this diauxic growth pattern has relatively slow growth during the first phase and faster growth during the second phase. The effect is attributed to laboratory conditions not reflective of habitats that influenced *E. coli* selection [68].

Gram-positive *B. subtilis* also prefers glucose and utilizes the PTS system for sugar transport, although the molecular components of CCR are different than in *E. coli*. cCCR in *B. subtilis* is mediated largely by negative regulation through a repressor protein expressed in the presence of glucose. The key cCCR components include catabolite control protein A (CcpA), phosphocarrier protein (HPr), HPr kinase (HPrK), and glycolysis intermediates fructose-1,6-bisphosphate and glucose-6-phosphate [36–38]. HPr plays two important roles. First, HPr participates in the phosphorylation cascade by transferring the phosphate group from PEP to glucose, forming glucose-6-P. Second, HPr is a regulator molecule which responds to glycolysis indicators fructose-1,6-bisphosphate

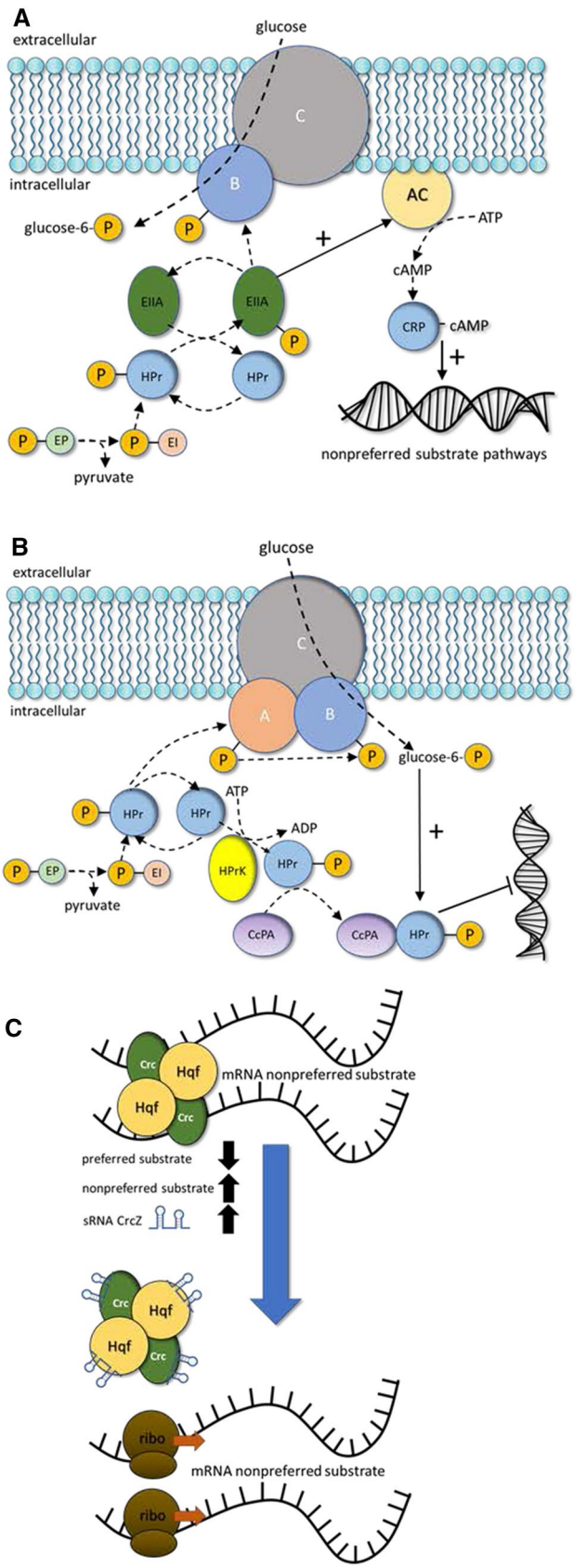

Fig. 2 Molecular components of carbon catabolite repression (CCR). ▶ **a** Major molecular components of classic carbon catabolite repression (cCCR) in Gram-negative *E. coli*. **b** Major molecular components of cCCR in Gram-positive *B. subtilis*. **c** Major molecular components of reverse carbon catabolite repression (rCCR) found in Pseudomonads. See main text for abbreviations and details

or glucose-6-phosphate to form a complex with CcpA. The CcpA–HPr complex, stabilized with glycolysis intermediates, can bind DNA, inhibiting expression of genes associated with non-preferred substrates [69–75]. Approximately 400 *B. subtilus* genes are influenced by CCR [76, 77].

Molecular mechanisms of rCCR

cCCR-mediated preference for glucose is not a universal strategy for generalist, glucose-catabolizing heterotrophs [9, 78, 79]. *Pseudomonas aeruginosa* and *Pseudomonas putida* consume preferred carbon substrates in nearly reverse order from *E. coli* and *B. subtilis*. They preferentially catabolize organic acids and amino acids before catabolizing glucose [8, 78, 80–82]. Study of rCCR lags the study of cCCR, highlighting a research area of opportunity.

Known rCCR mechanisms in Pseudomonads operate at the mRNA level; this is contrary to most cCCR microorganisms, which are governed by DNA-binding transcription regulators [83, 84]. RNA chaperone protein Hfq represses expression of catabolic targets for non-preferred substrates by binding directly to the 5′ end of the mRNA. Catabolite repression control (Crc) protein interacts with Hfq, creating a stabilized Hfq/Crc/mRNA complex, enhancing regulation. Hfq and Crc regulons have significant overlap [85–87]. Computational analysis of Crc-binding sites predicted 143 candidate genes in *P. putida* and 215 candidate genes in *P. aeruginosa,* while experimental hfq gene deletion studies identified 212 target genes in *P. fluorescens* [88–90]. Small regulatory RNAs (sRNA) act as antagonists to Hfq, binding the protein and relieving repression of the non-preferred catabolic pathways. *P. aeruginosa* has 1 characterized sRNA (CrcZ), while *P. fluorescens* and *P. putida* have two (CrcY, CrcZ), as does *P. syringae* (CrcZ, CrcX) [84, 91, 92].

Theoretical aspects of CCR

CCR is the collective result of many interacting molecular components. The complexity necessitates in silico representations for quantitative predictions. Decades of in silico CCR models exist, most focusing on *E. coli* [67, 93]. The earliest in silico models were primarily mechanistic, comprised of mass balances on enzymes, metabolites, and metabolic regulators, and were represented by ordinary differential equations. As a result, they were limited by the availability of

experimentally measured enzyme parameters and metabolite concentrations [67, 93]. These models were not well-suited to address the ecological rationale of CCR strategies. One exception is cybernetic modeling, which examines implicit aspects of resource investment into metabolic strategies and can be used to study diauxic growth [95]. The modeling technique requires a priori calibration data to identify cybernetic parameters which are then used to interpret pathway resource requirements; the cybernetic parameters do not necessary map explicitly to molecular components [96].

The omics revolution has generated huge data bases which have been leveraged by a metabolic modeling family known as stoichiometric modeling; two common types of stoichiometric modeling are flux balance analysis (FBA) and elementary flux mode analysis (EFMA) [97–100]. Stoichiometric models mathematically define the phenotypic capabilities of metabolic systems by accounting for the net transformation of all enzyme-catalyzed reactions as well as relevant abiotic reactions. The mass balances for all system components can be written as a set of linear equations when appropriate time scales are assumed; this negates the requirement for difficult to measure enzyme parameters. The modeling approach does require a minimal set of parameters such as biomass composition and cellular maintenance energy values [101].

A remarkable number of stoichiometric modeling studies have examined aspects of cCCR, including maximum growth rates, overflow metabolisms, and sequential use of substrates. Stoichiometric models define a mathematical solution space of all possible cellular phenotypes based on the linear mass balance equations; context specific flux distributions are identified using optimization criteria. The most commonly used optimization criterion identifies phenotypes that maximize growth rate, consistent with the basic tenets of cCCR [102–104]. All microbial systems are resource constrained and metabolic strategies that maximize fitness must competitively allocate limiting resources. Early applications of stoichiometric models examined limited accessibility of a catabolic resource, O_2, and identified metabolisms that maximize energy extraction from glucose while minimizing consumption of O_2 [99, 100, 105, 106]. Resource allocation into cCCR-relevant strategies has been a topic of many stoichiometric studies and has resulted in predictions of overflow metabolisms that secrete partially oxidized byproducts such as acetate even in the presence of electron acceptors such as O_2. Overflow metabolisms were for decades described as a wasteful metabolism that did not extract all the available energy from a substrate, but recently this view has changed [31]. Representation of resource scarcity in stoichiometric models has expanded to consider anabolic resources, and the simulations have provided ecological explanations for overflow metabolisms occurring at both fast growth rates during batch cultivation as well as slow growth rates during chemostat cultivation [107]. Numerous proxies for anabolic cellular resources have been described in *E. coli,* with each generating similar outcomes; metabolic fluxes are directed to maximize energy production from the limiting resource, which leads to partial oxidation of substrate and an overflow metabolism. Basically, when glucose is the preferred substrate, glycolysis is an essential pathway, while auxiliary metabolic routes are optional and thus, down regulated when an anabolic resource becomes scarce. Anabolic resource considerations have included (1) accounting of the moles of a resource (e.g., nitrogen, carbon, amino acid) required to synthesize a metabolic pathway relative to the catabolic efficiency of the pathway [31, 32], (2) the volume of the cytosol occupied by enzymes relative to the catabolic efficiency of the flux distribution [103, 104], and (3) the membrane surface area occupied by membrane complexes required for a cellular flux distribution [108, 109] as well as other representations of optimal proteome investment into metabolic fluxes [5, 110]. In silico metabolism representations have also expanded to account for not only metabolic pathways but also the enzymatic machinery required to synthesize RNA and protein [111–113]. The converging of numerous resource allocation problems onto similar phenotypes highlights common relationships between the different proxies regardless of the level of molecular details considered. Resource allocation into enzymes has also been used to justify the sequential catabolizing of glucose and lactose in a mixture. For example, glucose catabolism requires fewer enzymes, permitting larger concentrations of the enzymes and driving a faster growth rate than lactose catabolism [94, 114].

The ecological basis of rCCR is not well studied. Common hypotheses invoke the availability of substrates during the organisms' evolutionary histories. In many environments, such as soils, sediments, and plant surfaces, organic acids are plentiful byproducts of biomass degradation or plant secretions [7, 8]. However, these environments also contain sugars [115]. Organisms such as *P. aeruginosa* and *P. putida* are competent glucose catabolizers, but their regulation schemes do not prioritize the substrate. A 'gluttony' hypothesis has been proposed which postulates that Pseudomonads utilize a strategy of consuming substrates as fast as possible to deny the resources to competitors [90]. The hypothesis states the rCCR regulation is necessary to balance metabolic intermediates which could accumulate to inhibitory concentrations. The theory postulates the outcome of rCCR is not to select 'preferred substrates' but rather to repress substrates in a manner that balances metabolism. The theory does not explicitly address the sequential use of substrates or why glucose is not co-metabolized.

Few, if any, modeling studies explicitly address the basic tenets of rCCR such as the preference for organic acids over glucose, the lack of almost any overflow metabolism,

and a propensity to not always maximize growth rate in monoculture [9, 30, 116–118]. Resource availability limits bacterial productivity in most environments, so widely distributed bacteria such as Pseudomonads have surely been influenced by resource scarcity. The role of resource allocation to enzymes in rCCR organisms is unknown, but it generally contradicts theories postulated for *E. coli*. rCCR organism do not optimize investment into glycolysis, at the expense of respiration, resulting in an overflow metabolism; in fact, rCCR organisms catabolizing glucose do not typically exhibit an overflow metabolism and instead completely oxidize the sugar to CO_2 [118–120]. All Pseudomonads catabolize glucose using the ED pathway [118, 121]. The ED pathway minimizes investment into enzymes as compared to the higher cellular-energy yielding EMP pathway [2, 31, 32, 122], (Fig. 3) providing hints of potential resource allocation strategies in Pseudomonads. In silico representations of *P. aeruginosa* and *P. putida* metabolism using stoichiometric models used the maximization of growth optimization criterion to identify flux distributions [123–126]. rCCR organisms do not always select preferred substrates based on maximizing growth rates [30].

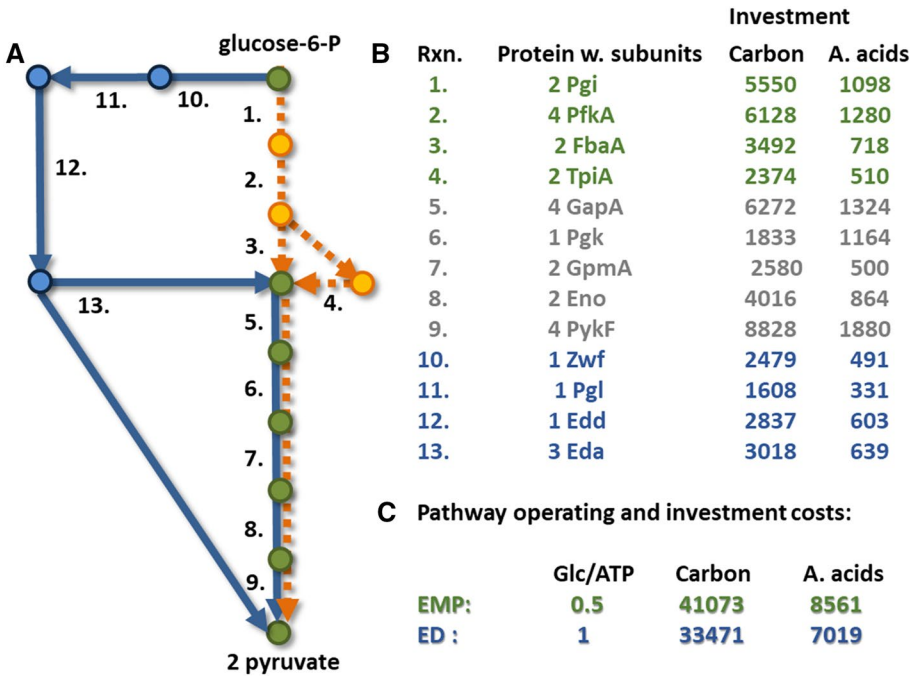

Fig. 3 Trade-off between resource investment into enzymes and energetic efficiency of the Embden–Meyerhof–Parnas (EMP) (orange lines) or Entner–Doudoroff (ED) (blue lines) glycolysis pathways. Shared components are colored green. Pseudomonads use the ED pathway to catabolize glucose; the ED pathway requires fewer resources to assemble but also extracts less cellular energy from glucose than the EMP pathway. Reaction numbers map to enzymes, protein subunit compositions, and the resources required to assemble functional enzyme based on *E. coli* protein sequences. Data from Carlson, 2007 [31], figure modified from Carlson and Taffs, 2010 [2]

Table 1 Summary of carbon catabolite repression (CCR) mechanisms and key molecular components in representative Gram-positive and Gram-negative microorganisms

Microorganism	Gram staining	Governing mechanism	Important components	References
E. coli	−	Positive induction of DNA transcription	EIIA-P, cAMP, CAP	[34, 35]
Bacillus subtilis	+	Negative regulation of DNA transcription	CcpA, HPr, HPrK	[36–38]
Lactobacillus brevis	+	Negative regulation of DNA transcription	CcpA, HPr, HPrK	[39]
Lactococcus lactis	+	Negative regulation of DNA transcription	CcpA, HPr, HPrK	[40]
Clostridium cellulyticum	+	Negative regulation of DNA transcription	CcpA, HPr, HPrK	[41]
Corynebacterium glutamicum	+	Negative regulation of DNA transcription	Ram A, RamB	[42, 43]
Acinetobacter baylyi	−	Repression of mRNA translation	Crc, Hqf, CrcZ	[7–9, 44]
Pseudomonas putida	−	Repression of mRNA translation	Crc, Hqf, CrcZ	[7–9, 44]
Pseudomonas aeruginosa	−	Repression of mRNA translation	Crc, Hqf, CrcZ	[7–9, 44]
Streptomyces coeiicoior	+	Biochemical state of glucose kinase	Glk	[45, 46]

CCR and social interactions

Microbial consortia are ubiquitous in nature, and competition for resources often predicts the outcome of microbial interactions [127–130]. Therefore, it is logical that global regulation strategies such as CCR are linked to social behaviors such as quorum sensing, biofilm formation, and virulence mechanisms including antibiotic or ROS sensitivity [131, 132]. Examples of the different social interactions for cCCR and rCCR organisms are summarized in Table 2.

Quorum sensing is an intercellular communication mechanism which enables coordination of cellular phenotypes across populations and space [133]. Quorum sensing involves the synthesis and secretion of small soluble molecules that accumulate in the environment. At critical concentrations, the soluble molecules are imported into the cells and initiate gene regulation. Quorum sensing is regulated by CCR [134–136] (Table 2). CCR-influenced quorum sensing is also required for other social behaviors including the development of mature biofilms [137, 138].

Biofilms are microbial communities encapsulated in self-produced polymer and are often associated with interfaces [139, 140]. Biofilms are social structures with high densities of cells (~ 300 g/L), up to two orders of magnitude higher than typical in vitro culture and many orders of magnitude higher than common environmental populations [141–143]. Metabolite transport in biofilms is primarily mediated by diffusion, and high cell density and high cellular activity create mass transfer limitation and nutritional gradients [82]. Biofilms are argued to promote the cellular interaction strategy of altruism [131, 144]. Recent research demonstrated a remarkable substrate 'flux time-sharing' and metabolic oscillations used by altruistic *B. subtilis* cultures to partition resources between cells on the periphery of a biofilm and cells deep within a biofilm [145, 146]. The cells on the interior and exterior of the biofilm oscillate their activity in a coordinated manner, taking turns metabolizing the limiting substrate, which resolves conflict for the substrate and ultimately enhances community growth.

Virulence mechanisms refer to a broad range of cellular strategies that, in a host, enhance resource acquisition, growth, and persistence and are often associated with CCR [7, 147]. Common examples of virulence mechanisms include antibiotic tolerance, production of enzymes such as catalase, superoxide dismutase, and other enzymes for either producing or mitigating ROS, secretion of resource acquisition molecules such as siderophores, proteases, lipases, elastinases, or haemolysins, as well as the quorum sensing and biofilm phenotype mentioned previously (Table 2). Virulence mechanisms, such as antibiotic tolerance, are typically studied from the perspective of monocultures and medical challenges [148–150]. Antibiotics in microbial environments precede human use; these agents are part of natural microorganism interactions used to influence the distribution of species in consortia; secretion and degradation of antibiotics can stabilize consortia [151, 152]. Antibiotic and ROS susceptibility is well documented as a function of CCR [153, 154].

Table 2 Summary of social behaviors regulated by carbon catabolite repression (CCR) mechanisms in both classic (c) and reverse (r) CCR utilizing microorganisms

	Microorganism (gram stain)	CCR	Social behavior linkage to CCR	
Biofilm formation	*E. coli* (-)	c	cAMP-CRP regulates csgD and biofilm formation	[155, 156]
	P. aeruginosa (-)	r	Crc is required for aerobic biofilm formation	[157]
	P. aeruginosa (-)	r	CrcZ cross-regulation controls anaerobic biofilm formation	[158]
	P. aeruginosa (-)	r	cbrB is linked to biofilm formation and dispersal	[159]
	S. aureus (+)	c	CcpA regulates biofilm formation	[160]
	S. *epidermidis* (+)	c	CcpA regulates biofilm formation	[161]
Quotum sensing	*E. coli* (-)	c	Cocrystal structures of LsrK and HPr establishes link between CCR and QS	[162]
	E. coli (-)	c	cAMP-CRP influence AI2 synthesis and uptake	[163, 164]
	P. aeruginosa (-)	r	Lon and CIpXP proteases link CCR and QS	[136]
	S. aureus (+)	c	Ccpa upregulates Agr which is an autoinducing QS peptide	[165]
Stress tolerance	*P. aeruginosa* (-)	r	Hfq-dependent antibiotic susceptibility	[148]
	P. aeruginosa (-)	r	Oxidative stress tolerance orchestrated by Crc	[149]
	E. coli (-)	c	Acetate CCR regulates biofilm formation and virulence	[150]
	S. aureus (+)	c	CCpE control virulence factors including alpha toxin	[166]
	P. syringae (-)	r	Crc influences virulence, oxidative tolerance, and biofilm formation	[167]

Division of labor and resource partitioning in microbial consortia

Interactions between microorganisms with similar catabolic preferences are often antagonistic [24]. Resource partitioning through mechanisms such as division of labor or reciprocal prioritization of substrates can mitigate direct competition [22, 23, 128, 168–170]. cCCR and rCCR utilizing organisms are often found to coexist in medical environments, including chronic wounds and cystic fibrosis lungs [171]. rCCR organism *P. aeruginosa* has been isolated from 15 to 80% of chronic wounds, while cCCR organism *S. aureus* has been found in more than 90% of chronic leg ulcers [172–178]. *S. aureus* and *P. aeruginosa* are excellent biofilm formers and often isolated together [179, 180]. Synergistic interactions in polymicrobial infections can lead to greater virulence likely through enhanced resource acquisition and greater efficiency of converting resources into new biomass. For instance, wounds colonized by polymicrobial consortia have more negative patient outcomes than wounds colonized by monocultures [173, 177, 181, 182].

CCR regulates a wide range of social behaviors and likely modulates division of labor or reciprocal substrate prioritization (Table 2). cCCR and rCCR consortia represent an intuitive division of labor, whereby the rCCR organism consumes byproducts of the cCCR metabolism removing metabolites that could be a thermodynamic constraint on metabolism as well as inhibitory [183] (Fig. 1). This positive feedback mechanism would enable consortia to increase biomass productivity by more completely depleting available substrates than a monoculture [184, 185] (Fig. 3).

rCCR microorganisms prefer non-fermentable substrates such as organic acids necessitating the presence of a terminal electron acceptor, such as O_2 [116]. The availability of O_2 is a fitness challenge especially in diffusion-limited biofilms, where other organisms could consume the catabolic substrate. *P. aeruginosa* is a very competitive microorganism with mechanisms to acquire limited resources such as O_2. First, *P. aeruginosa* utilizes quorum sensing and other community surveillance tactics to assess and regulate the type of competitive strategies employed [21, 176, 186–188]. These strategies involve the secretion of phenazine moieties such as pyocyanin, quinolones, and cyanide. The phenazine- and quinolone-based molecules have numerous social and resource acquisition activities including antibacterial properties, biofilm formation, iron chelation, cellular communication, extracellular electron transport and the production of ROS [189–193]. These molecules, along with cyanide which is also secreted by *P. aeruginosa*, can inhibit respiration in neighboring cells [188, 194]. Collectively, these mechanisms would modulate the phenotypes of nearby cells, forcing them into a fermentative metabolism which partitions O_2 for *P. aeruginosa* respiration, while at the same time producing organic acids, a preferred carbon source. Long-term exposure to quinolones selects for small colony variants (SCVs) of *S. aureus* which have impaired respiratory activity and utilize primarily fermentative metabolisms [180, 188, 195].

Direct measurement of biomass productivity in natural consortia with cooccurring cCCR and rCCR microorganisms is difficult; artificial, in vitro culturing conditions often result in imbalanced resource partitioning, competition for resources and antagonism [181, 196]. Quantitative biomass productivity results are available for both evolved and synthetic systems that behave analogously to a cCCR + rCCR consortium. These systems have defined division of labor consisting of a primary, glucose-catabolizing population and a secondary, byproduct catabolizing population. The primary population prefers glucose, maximizes growth rate, and utilizes an overflow metabolism with the secretion of acetate and other byproducts. The byproduct population catabolizes the overflow byproducts and increases system productivity via two mechanisms. First, it modifies the local environment by removing acetate, which is a potent growth inhibitor that also lowers biomass yields on substrate, and secondly, the population utilizes non-preferred substrates that would otherwise be unutilized by the cCCR microorganism. The system design is simple but has a remarkable effect on system productivity. The in vitro consortium with evolved division of labor has 15% improvement in biomass productivity relative to the original monoculture during planktonic growth [197, 198]. The synthetic consortium with engineered division of labor has a 20% improvement in biomass productivity during planktonic growth and a 50% increase in biomass productivity during biofilm growth [199]. Both the evolved and engineered systems utilize substrate more effectively, driving glucose to lower concentrations [197–199]. The increases in biomass productivity can be explained by an enhanced return on carbon investment derived from the kinetic effect of division of labor, the removal of inhibitory byproducts which promotes more complete depletion of substrate, and the catabolism of non-preferred substrates that would otherwise be wasted. The section below provides a quantitative analysis of the effects.

Theoretical analysis of division of labor enhancing biomass productivity

Enzyme flux is realized by investment into both enzymes and substrate pools. There is an optimal relationship between enzyme and substrate concentrations that minimizes the total investment necessary to drive a flux [107, 200–203]. Cross feeding consortia utilizing division of labor can have a better functional return on investment based on the properties of Michaelis–Menten kinetics. An example is presented which

demonstrates a reduced total requirement for carbon with cross feeding between two specialist consortium members as compared to two generalist microorganisms. The aggregate specialist consortium and generalist system perform the same net transformation of glucose to CO_2 and both systems consume glucose at the same total rate. For the quantitative example, enzymes from glycolysis are represented by properties of the Pgi enzyme and enzymes from the tricarboxylic acid (TCA) cycle are represented by the properties of the FumA enzyme. Irreversible Michaelis–Menten kinetics are assumed for the single substrate reactions; enzyme amino acid sequence and subunit composition for *E. coli* are used to calculate carbon investment into enzymes and substrates. Figure 4 details the enzyme parameters, overall biochemical pathways, and the cross feeding scheme based on a cCCR and rCCR metabolism.

The generalist system is comprised of two cells each operating a complete oxidation of glucose and each consuming glucose at a specific rate of 1 mmol glucose g cdw^{-1} h^{-1}. The generalist cells have 21 considered enzymatic steps. The specialist consortium is also comprised of two cells with the first cell consuming glucose at a rate of 2 mmol glucose g cdw^{-1} h^{-1} and secreting lactate at 4 mmol lactate g cdw^{-1} h^{-1}. The first cell has 10 considered enzymatic reactions. The second cell consumes the lactate at the same rate it is secreted. The second cell has 13 considered enzymatic reactions. When carbon investment into both enzymes and substrate pools is considered, the cross feeding system requires ~ 4% smaller investment to operate the same net transformation even though it contains two more enzyme-catalyzed steps than the generalist system. This system is analogous to cross feeding between *S. aureus* and *P. aeruginosa*. The reduced resource requirement is available for enhanced growth, providing an explanation for the enhanced biomass productivity.

The catabolism of overflow byproducts removes inhibitors, which improves overall consortium productivity, as mentioned above. This effect has been quantified in a recent stoichiometric modeling study that examined biofilm growth of an engineered, division of labor consortium which cross feeds acetate [204]. The model incorporated the genome-encoded metabolic potential of the two microorganisms, the diffusion-based transport of metabolites in the biofilm, and considered the inhibitory properties of the exchanged acetate (Fig. 5). The simulations captured the enhanced biomass productivity, approximately 50% improvement, observed in the corresponding experimental study based on the removal of the inhibitory acetate [199]. This modeling study did not account for resource investment into intracellular enzymes and metabolites highlighting how each mechanism in isolation can enhance productivity. Integrating both mechanisms into a computational representation is anticipated to further capture in situ mechanisms of productivity.

Conclusions

Carbon catabolite repression is the result of natural selection within dynamic environments. Two common forms of carbon catabolite repression, classic carbon catabolite repression and reverse carbon catabolite repression have broad global distributions in natural microbial populations and have near mirror-image preferences for substrates. These metabolic strategies are typically studied from the perspective of monocultures. Here we present a consortia-based argument for the two metabolic strategies based on reciprocal social interactions including quorum sensing, biofilm formation, and virulence mechanisms. Appropriate ecological interpretation of global regulation schemes can open many new avenues of research leading to a greater understanding of the microbial world and its uses in agriculture, industry, and medicine. Box 3 highlights interesting, unresolved questions regarding rCCR

Box 3: Unresolved CCR questions.

1) What ecological strategy explains the intracellular resource allocation patterns observed during a rCCR?

2) rCCR strategies are respiration-centric so how does the phenotype allocate cellular membrane surface area relative to a cCCR microorganism utilizing an overflow metabolism?

3) How does the distribution of cellular membrane surface area allocated to enzymes change during division of labor and cross feeding?

4) *P. aeruginosa* is a proficient biofilm former and operates a respiration-centric rCCR metabolism which sets up an important ecological question, does the *P. aeruginosa* biofilm phenotype also employ a rCCR strategy even though biofilms are limited by O_2 diffusion?

5) How do distributions of cCCR and rCCR microorganisms change with time in chronic wounds and CF lungs? Does early colonization favor a cCCR metabolism while evolved communities select for interacting consortia?

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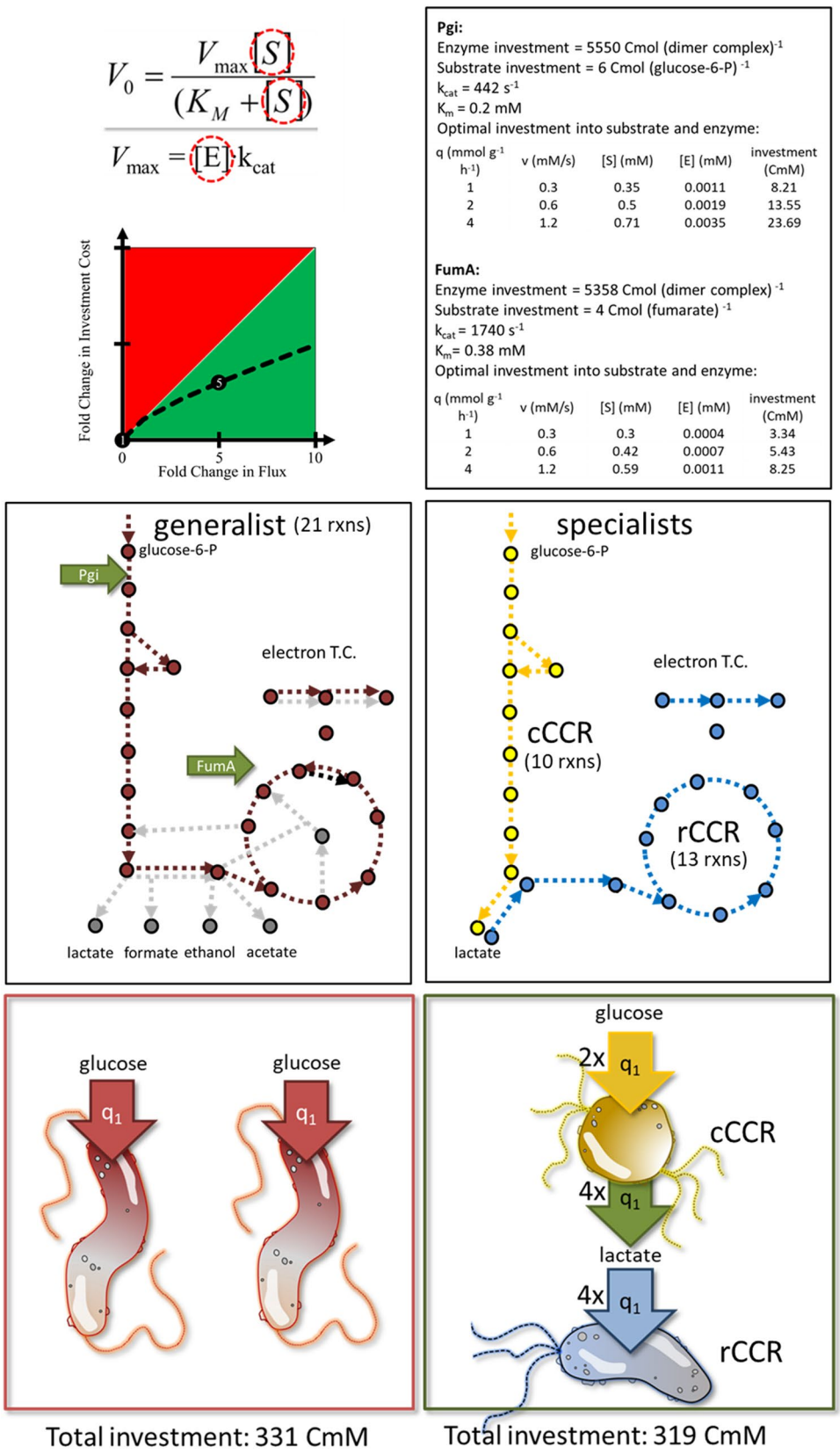

Fig. 4 Illustration of enhanced functional return on carbon investment in a cross feeding consortium relative to a generalist system. Enzyme flux requires investment into both enzyme and metabolite pools which can be optimized to reduce total cellular investment. Operating enzymes at higher fluxes represents a better functional return on resource investment (e.g., aggregate carbon atoms in enzyme and substrate). Analysis considers Michaelis–Menten-type kinetics, 2 cells using a generalist strategy, or 2 specialist cells cross feeding. The overall glucose flux and glucose transformation are the same for both scenarios. Glycolysis reactions are represented by the enzyme Pgi, while tricarboxylic acid (TCA) cycle enzymes are represented by the enzyme FumA. Enzyme values are from *E. coli* and obtained from Brenda and EcoCyc. The specialist consortium requires a smaller investment of carbon to attain the same flux and transformation as the generalist system. Specific glucose uptake rate (q_1) was set to 1 mmol glucose g cdw^{-1} h^{-1}. Portion of figure modified from Beck et al. 2016 [200]

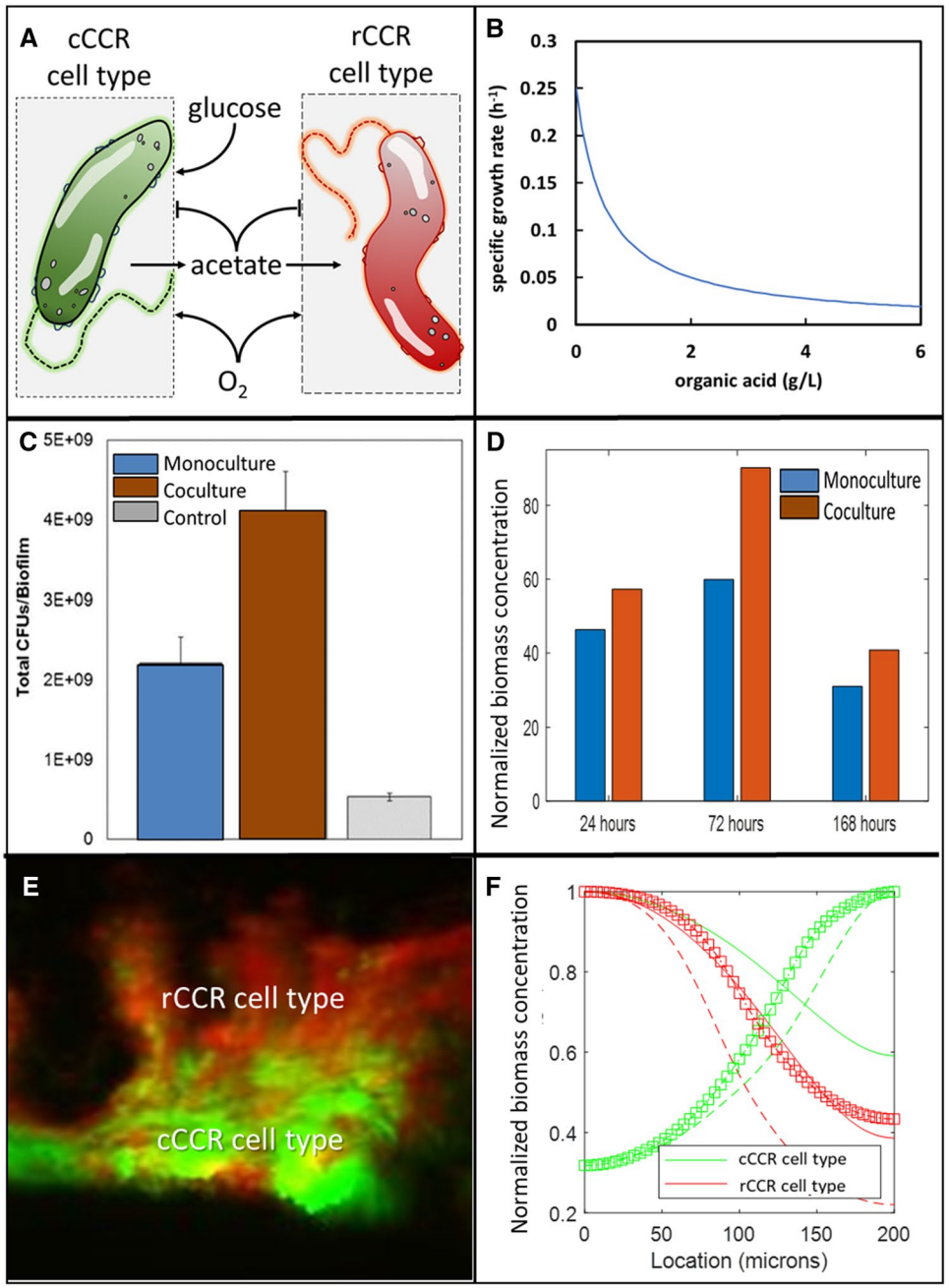

Fig. 5 Role of byproduct inhibition in cross feeding consortia growing as biofilms. **a** Schematic of metabolic interactions between cCCR- and rCCR-cell types. **b** Example of the inhibitory properties of an organic acid as a function of concentration. **c** *In vitro* data for an engineered, cross feeding consortium growing as a biofilm. **d** In silico prediction of biomass productivity for an engineered consortium growing as a biofilm. **e** Micrograph of a cryosectioned biofilm comprised of an engineered consortium, rCCR-cell type expressing rfp, cCCR-cell type expressing gfp. **f** *In silico* predictions of cell-type spatial distributions within a biofilm. Portions of the figure are modified from Patel et al. 2019 [204] and Bernstein et al. 2012 [199]

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