Synthetic biology

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Synthesizing cellular LOGIC

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Engineering synthetic tools that facilitate decision-making in mammalian cells could enable myriad biomedical applications. Researchers have now developed a new system of inducer-controlled transcription factors to facilitate synthetic decision-making (LOGIC) in human cells based on modular protein-fusion cascades.

Precise and coordinated control of gene expression is a grand challenge that is faced by synthetic biologists[1]. One of the overarching goals of the field is to develop regulatory tools that will allow researchers to match or exceed the level of biological complexity that nature achieves[2]. Despite over two decades of research focused on creating synthetic genetic circuits, substantial hurdles remain — particularly with regard to engineering programmed gene expression in human cells. In this issue of *Nature Chemical Biology*, Bertschi et al.[3] provide a new framework for creating genetic circuits in human cells based on rationally designed networks of interacting transcription factors (TFs) that can be controlled with orthogonal chemical inducers by way of a technology called LOGIC (large orthogonal gates based on inducer-controlled cascades of protein fusions).

The use of inducible TFs has been widely exploited to confer decision-making (Boolean logic) in prokaryotic chassis cells[4,5], but their adaptation to mammalian cells has proven challenging. Boolean logic is a mathematical structure that enables the calculation of a result as either TRUE or FALSE and is the foundation for modern computing and device structure. In prokaryotic chassis cells, Boolean logic is achieved via the systematic arrangement of inducible promoters coupled with cognate transcription factors that process disparate small molecule inputs. The application of Boolean logic in mammalian cells has proven

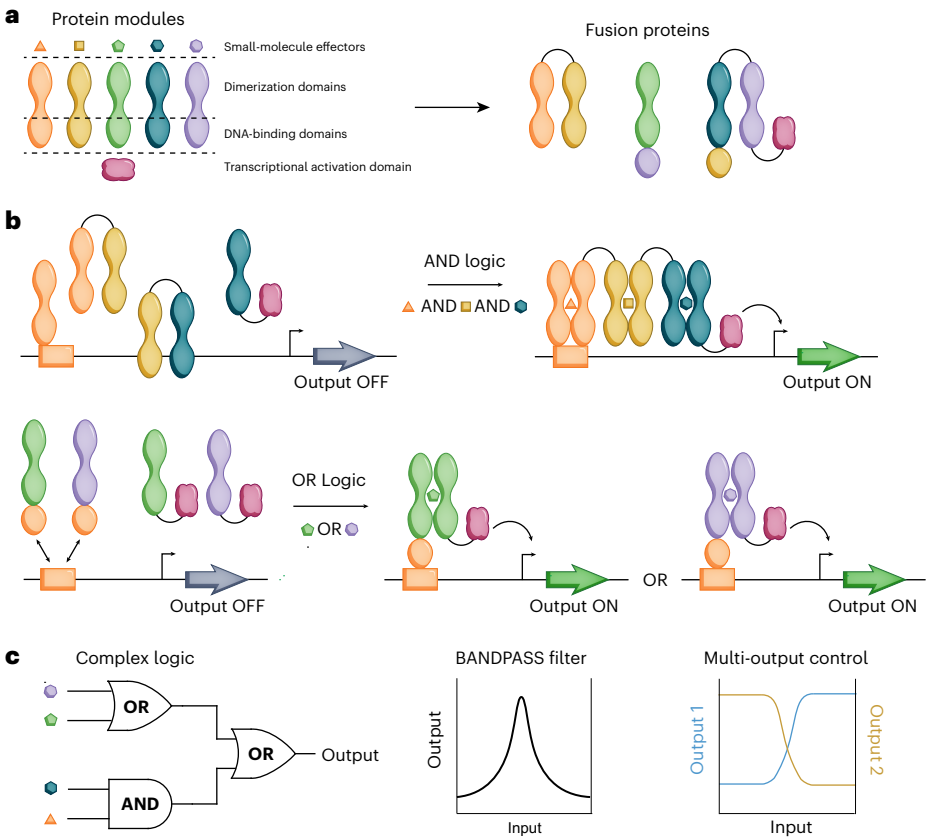

Fig. 1 | Exemplars of human LOGIC synthetic circuits. a, The components of the LOGIC system and relevant domains. Modular fusion proteins are constructed from transcription factors and transcriptional activation domains. **b**, AND gates (top) and OR gates (bottom) can be constructed based on fusion proteins and inducible dimerization patterns **c**. LOGIC technology enables multi-input genetic circuits such as (A OR B) OR (C AND D) (left), bandpass devices (middle) and multi-output control (right).

to be more challenging (relative to bacteria) owing to the complexity of eukaryotic promoters and the intricate ensemble of proteins necessary to facilitate gene regulation. To develop the LOGIC platform for mammalian genetic circuit design, the authors began by identifying TFs that would dimerize in the presence of their cognate effector. First, the TetR protein — which binds to its DNA operator sequence in the absence of doxycycline — was fused to one of several candidate TFs. The resulting fusion protein module was expressed in human embryonic kidney (HEK) cells containing a TetR-binding promoter upstream of a human placental secreted alkaline phosphatase (SEAP) reporter gene. A second module was simultaneously expressed and consisted of the same candidate TF fused to a transcriptional activator domain (Fig. 1a). The corresponding modules were then tested for their ability to activate gene expression when the effector of the candidate TF was provided in the growth medium. The authors found that several TFs could be triggered to form heterodimers and subsequently activate SEAP expression. Given that TetR dissociates from its operator in the presence of doxycycline, the resulting synthetic expression systems displayed NIMPLY Boolean logic (that is, output is only on in the presence of one input), which can be useful for modulating gene expression via the repeated addition of inducers. Logical operations of this sort could prove to be valuable in gene therapy and advanced stem cell differentiation — that typically rely on extrinsic signal control — as the NIMPLY operation can facilitate OFF to ON to OFF gene regulation via repeated addition (opposed to dilution or signal degradation), which results in faster response times[6].

The modular design of the switchable fusion proteins allowed for engineering multi-input gene circuits by way of inducible cascades of heterodimers. Namely, by expressing multiple modules that consisted of different TF fusions, the researchers were able to create multiple-input AND gates that were dependent on up to 5 unique inputs (Fig. 1b). The scope of the fusion protein switches was extended to the fusion of a dimer-forming TF to a DNA-binding domain (DBD), which alone allowed for the localization of the module to a specific operator. This additional function facilitated effector-mediated triggering of heterodimerization and subsequent activation of a promoter in the form of a single-input ON-switch. Moreover, an AND gate operation could be readily converted to an OR gate operation by co-expressing multiple unique modules that have the same DBD fusion. Specifically, given that multiple fusion modules can recognize the same operator, induction with any of their effectors creates a heterodimer complex that is capable of activating the transcription of the output gene (Fig. 1b).

To demonstrate advanced synthetic information processing via LOGIC, the authors designed a biotic BANDPASS filter (Fig. 1c). Here, the TetR-VanR ON-switch was paired with a previously developed VanR OFF-switch — that is, VanR fused to a transcriptional activator domain, which dissociates from its cognate promoter in the presence of inducer. Having each switch control dimerizing partners from an orthogonal ON-switch, the authors were able to create an operation that only allowed expression of the SEAP reporter at intermediate concentrations of inducer — that is, a BANDPASS filter (Fig. 1c). While similar devices have been engineered and implemented in prokaryotic systems with a single transcription factor[6], Bertschi et al. posited that this iteration is among the most compact designs for mammalian cells. The utility of a BANDPASS filter can be appreciated in its relation to cellular differentiation, as local chemical gradients often result in cellular patterning.

Although challenges remain in engineering mammalian transcription-based circuits, this new framework for inducible protein interaction-based genetic circuit design could increase the complexity of biological programs implemented in human cells. Engineering synthetic biological functions more efficiently and reliably will allow for more precise control over cellular systems, ultimately leading to technological advances in synthetic biology and its potential applications. Decision-making programs like the ones developed by Bertschi et al. could have exciting applications in gene therapies, stem cell differentiation and tissue engineering. Future work to develop additional LOGIC programs could expand the computational capacity of genetic circuits in mammalian cells. If expanded to other cell types, and combined with complementary technologies based on RNA sensing[7,8] and protein sensing[9,10], the field could step closer towards achieving its goal of emulating natural biosystems — for example, the development of fully synthetic pluripotent stem cells.

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Competing interests

The authors declare no competing interests.