Accentuate the positive and eliminate the negative
Commentary
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“Negative Capability, that is, when a man is capable of being in uncertainties, mysteries, doubts, without any irritable reaching after fact and reason.” Keats

Plant developmental signals all appear to work by countering the suppression of a response, that is, by opposing the action of negative regulators. Do negative controls rule the signalling pathways used?

The search for genes encoding proteins necessary for connecting signals to development in plants has thrown up a surprising number of negative regulators. SPY, GAJ and RGA for gibberellin signals, KRA1 for abscisic acid signals, SIK1 for auxin signals, SPA1 for UV/blue signals and COP1/DET/FT5 for light signals generally. Are these the first signs of an emerging principle of control of plant development: that signals act by down regulating a default mode adopted in the absence of signal? I suspect not.

There are enough candidate positive regulators to suggest that the negative regulators don’t have a monopoly: COP/DET/FUS and RGA for auxin signals, the Petunia TRA1 mutants showed decreased sensitivity for cytokinin signals, and IAA17 for auxin signals, the Petunia TRA1 for cytokinin signals, and B5 for light signals. The phenotypes resulting from mutation in these genes reflect the complexity of the web of interaction disturbed as a result.

The same signal transduction component can be both a negative and a positive regulator because of network dynamics

Whether a gene product is a positive or negative regulator can be context-dependent. Responsive specifically to cytokinins, tra1 mutants showed decreased sensitivity for some responses, increased for others [1]. Although IAA1 is a positive regulator for the root growth response to auxin, the phenotypes of both gain-of-function and loss-of-function alleles show the same gene is a negative regulator of auxin signals for germination and lateral root formation, while similar member of the same family, IAA17, is a positive regulator for the latter response [2].

How can the same protein be both a negative and a positive regulator of signal response? One possible explanation lies in the nature of the pathways linking signal to response. The ‘wiring’ analogy isn’t helpful. Although we’re well aware it’s incorrect, I suspect most of us still think intuitively of signals moving through otherwise empty channels as pulses of positive stimulus from plasma membrane to transcription complexes. As is often pointed out (usually by biochemists), the real signal transduction apparatus of the cell is more like an interconnected web of metabolic pools each in a state of furious, unceasing turnover, the level of signal shifting with the balance of generation and destruction or conversion. Loss of any one of the connections between pools will result in part of the web downstream becoming less accessible to stimulus, but the upstream accumulation may spill over sideways, activating sections used by other responses. It’s easier to visualise for G-proteins because the biochemistry of turnover is familiar. In mammalian systems, by speeding up G-protein de-activation RGS (regulators of G-protein signalling) proteins speed up G-protein de-activation and as a result negatively regulate long lasting signals controlling slow, irreversible processes. Paradoxically, the same proteins activate signals in the millisecond to seconds range, probably because increased de-activation speeds up G-protein turnover so that this signalling system is now fast enough to be accessed by brief signals [3]. There is a plant signalling component ABI1, with similarly paradoxical characteristics. ABI1 is a protein phosphatase that acts as a positive regulator of abscisic acid signalling in guard cells, carrying out fast, reversible, physiological responses, but a negative regulator for slower abscisic acid-dependent gene expression, which is positively regulated by the opposing dephosphorylating protein Ca2+-dependent protein kinase ATCIPK1 [4]. Is it possible that ABI1 works in guard cells by tuning signalling kinetics like RGS does, in this case by shortening the time course of the turnover of phosphorylated proteins? Plant responses to signals are almost all slow and irreversible, that is to say developmental as opposed to physiological, usually with prolonged presentation times to allow cross- and multiple-checking, and this may be a reason for a more extensive use of negative regulators in their control.

Even transcription factors like, the IAA proteins are in a state of dynamic turnover, the control of which is clearly critical for auxin signalling [5]. It’s less easy to visualise how closely related transcription factors like IAA3 and IAA17 can exert opposite effects on the same response, but the Smad proteins of animals are a good example of this sort of paradox. Smad proteins are positive regulators that, on phosphorylation by a receptor kinase, activate transcription. A subfamily of Smads, the anti-Smads, act as negative regulators of the same responses, probably by generally getting in the way. Anti-Smads are themselves signal-induced and so provide negative feed-back loop turning of transduction dynamics [6].
Negative regulators may have been favoured by the selective advantage of negative regulators over positive regulators for developmental signaling in plants. If not a monopoly, is there still a preponderance of negative regulators over positive regulators for developmental signaling? Certainly easier to find the restoration or enhancement of response phenotype resulting from a lesion in a negative regulator. Cytological markers form the basis of non-selective screens, and amongst the resulting haul of new signallling component genes the honours look to be evenly divided between negative and positive regulators. \textit{AGE1} and \textit{AGE2} were picked up because they negatively regulate auxin-inducible-promoter-driven GUS. Although \textit{AGE2} negatively regulates \textit{IAA1} and \textit{IAA12}, \textit{AGE1} seems to be a positive regulator of \textit{IAA1}, \textit{IAA4} and \textit{IAA8} [7]. In a Godzillan scale [8] screen of more than 300,000 mutagenised transgenic seedlings, the expression of a promoter responsive to abscisic acid, low temperature and osmotic stress was monitored using luciferase as reporter. Of 103 lines in which response to any of these signals had been altered markedly, 45 appear to represent a lesion in a positive regulator, and 49 in negative regulators of signallling.

Because signals from different receptors are correctly routed to different destinations via common components, selectivity is a feature of the web not individual regulators. The same results provide a unique overview of the ‘pathways’ used by three signals. Because the promoter contains motifs that confer specific inducibility by abscisic acid only, and by osmotic stress and low temperature but not abscisic acid, the expression of luciferase was tuned out by some feature of the dynamics of the net, a feature that delay or prevent flowering are negative regulators. The problem is that our anthropomorphise; the real world is frequently counterintuitive. For long day plants like \textit{Arabidopsis}, flowering is the default mode. They constitute alternatives, and darkness may well lead to activation and light to repression of the genes whose expression generates the etiolated phenotype — but who’s looking? Returning to the issue of positive and negative regulation, might not mutants with a phenotype indicating the lesion is in a gene for a negative regulator of light signals turn out to have lost a positive regulator of dark ‘signal’? Isn’t this just what the brassinosteroid biosynthesis mutant \textit{abi2} is? Positive and negative: as descriptions of developmental control they’re interchangeable. It all depends on your particular standpoint.

For those who work on the induction of flowering, genes that delay or prevent flowering are negative regulators. Intuitively, flowering is an active step, a move into maturity, progress, so genes that facilitate the transition are described as positive regulators. The problem is that our particular ‘standpoint’ is anthropocentric, that when we rely on our intuition to judge the validity of our ideas, we anthropomorphise; the real world is frequently counterintuitive. For long day plants like \textit{Arabidopsis}, flowering is the default mode and the vegetative state a precarious diversion that can only be imposed by sustained, repeated exposure to (delicious irony) a dark signal. Negative regulators of flowering are equally, perhaps more, likely to be positive regulators of the vegetative state.
To sum up, it is misleading to think of signal and response as connected by a ‘chain’ of cause and effect, or by a ‘pathway’ of signal transduction, or by ‘hardwiring’. A fluid net is probably closer to the reality. A developmental response is a shift between alternative states in one particular direction. The influence of a signal on the response is mediated by dynamic changes in interconnecting components. Investigators like to designate the function of a component as ‘negative’ if a decrease in its activity promotes the response, ‘positive’ if such a decrease inhibits or reverses the response, but these terms have no absolute status. For other responses, or the same response in the reverse direction, a negative regulator may be positive, a positive regulator negative. We shouldn’t expect to find negative regulation predominating. We don’t.

Finally, we should be aware that ‘positive’ and ‘negative’ are value-loaded. The prevailing ethic approves of growth and progress and disapproves of stasis. In the investigator’s mind progress to the next stage, growth and more of anything will tend to be thought of as positive; remaining at the same stage, arrest, dormancy, and less of anything will be considered negative. With this mind set, almost any form of control carries net negative connotations.

A master of the counter-intuitive, who drew what he knew and not what he saw, has the last word.

‘Every positive value has its price in negative terms.’ Picasso

References