Elucidating the regulation of complex signalling systems in plant cells

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Abstract

The pollen tube represents a model system for the study of tip growth, and the root provides a valuable system to study gene and signalling networks in plants. Here, using the two systems as examples, we discuss how to elucidate the regulation of complex signalling systems in plant cells. First, we discuss how hormones and related genes in plant root development form a complex interacting network, and their activities are interdependent. Therefore their roles in root development must be analysed as an integrated system, and elucidation of the regulation of each component requires the adaptation of a novel modelling methodology - regulation analysis. Second, hydrodynamics, cell wall and ion dynamics are all important properties that regulate plant cell growth. We discuss how regulation analysis can be applied to study the regulation of hydrodynamics, cell wall and ion dynamics, using pollen tube growth as a model system. Finally, we discuss future prospects for elucidating the regulation of complex signalling systems in plant cells.

Key words: root development, hormonal crosstalk, pollen tube growth, coupling of hydrodynamics, cell wall and ion dynamics, regulation analysis, regulation coefficients.

Complexity of signalling systems in plant cells

The term 'regulation' has been frequently used when plant signalling systems are studied. However, the exact meaning of regulation and how to elucidate the regulation of plant signalling systems need scrutinising. The pollen tube represents a model system for the study of tip growth [1], and the root provides a valuable system to study gene and signalling networks in plants [2]. Here, using the two systems as examples, we discuss how to elucidate the regulation of complex signalling systems in plant cells.

Hormone signalling systems coordinate plant growth and development through a range of complex interactions. The activities of auxin, ethylene and cytokinin depend on cellular context and exhibit either synergistic or antagonistic interactions. Additionally, auxin is directionally transported through plant tissues, providing positional and vectorial information during development [3]. Hormones and the associated regulatory and target genes form a network in which relevant genes regulate hormone activities and hormones regulate gene expression [2, 4-6]. For example, expression of the *POLARIS* (*PLS*) gene of *Arabidopsis*, which transcribes a short mRNA encoding a 36-amino acid peptide, is repressed by ethylene and induced by auxin [7,8]. It was also shown that *pls* mutant roots are short, with reduced cell elongation, are hyper-responsive to exogenous cytokinins and show increased expression of the cytokinin inducible gene, *ARR5/IBC6*, compared with the wildtype [7]. On the other hand, in the *pls* mutant, auxin concentration decreases, cytokinin concentration increases and ethylene production remains approximately unchanged [7-9]. In the *PLS* overexpressing transgenic *PLOSox*, auxin concentration increases, while ethylene production remains approximately unchanged. In the ethylene resistant *pls etr1* double mutant, auxin concentration is approximately recovered to the same level as that in wild-type seedlings [7-9]. Moreover, immunolocalization studies revealed that both PIN1 and PIN2 protein levels increase in the *pls* mutant, and decrease in *PLSox*. In the ethylene-insensitive *etr1* mutant, PIN1 and PIN2 levels are lower than those in wildtype. In addition, the double mutant *pls etr1* exhibits reduced PIN1 and PIN2 levels compared to *pls* and slightly lower PIN1 and PIN2 levels compared to wildtype [10]. Therefore, PLS and PIN1/PIN2 and three hormones (auxin, ethylene and cytokinin) form an interacting network, in which expression of *PLS* and PIN1/PIN2 levels regulate auxin, ethylene and cytokinin responses, which in turn regulate expression of PLS and PIN1/PIN2 [10]. Therefore, a key question is how to elucidate the regulation of such complex interacting signalling and gene expression systems in plant root development.

In addition, experimental information accumulated for many years indicates that hydrodynamics, cell wall and ion dynamics are important properties regulating plant cell growth, with much evidence generated from studying pollen tube growth. For example, experimental manipulation of tip-associated calcium ion gradients can result in re-polarisation [11], suggesting an intimate relationship between ion flux and pollen tube growth. Moreover, the stiffness of the cell wall is inversely correlated with growth rate, and there are no rapid and large-scale turgor changes during growth [12,13]. Therefore, cell wall properties are important in regulating pollen tube growth [12,13]. Furthermore, hypertonic and hypotonic osmolarity has been shown to cause the pollen tube apical area to shrink and swell respectively, and these changes correspond to the doubling and halving, respectively, of growth rate oscillatory periods compared to the oscillatory period of the isotonic growth condition [14-17]. Therefore, it was suggested that growth rate oscillations in pollen tube are regulated by hydrodynamics [14-17]. Since hydrodynamics, cell wall and ion dynamics are all important properties regulating pollen tube growth, an important question for

understanding pollen tube growth is how to elucidate the regulatory roles of hydrodynamics, cell wall and ion dynamics.

One of the common features of the signalling systems in root development and pollen tube growth is that change in one signalling component leads to change in other signalling components. Therefore, elucidation of the regulation in these signalling systems should take this feature into account.

Regulation analysis

Regulation analysis for a metabolic pathway quantitatively dissects the extent to which the flux through an enzyme is regulated by changes in metabolic level or by changes in gene expression [18-21]. The principle of regulation analysis was described by Rossell et al. [18]. Enzyme rate equations are usually described by eq 1:

$$v = v(e, X, K) = f(e) \bullet g(X, K) \qquad \text{(eq 1)}$$

where v is the rate, e is the concentration of enzyme, X is a vector of concentrations of substrates, products, and other metabolic effectors, and K is a vector of constants parameterizing the strength with which the enzymes interact with their substrates, products, and allosteric effectors [18].

Michaelis-Menten enzyme rate equation (eq 2) is a simple example of eq 1:

$$v = e\frac{k_{cat}X_S}{k_m + X_s} \qquad \text{(eq 2)}$$

where e is the concentration of enzyme, X_S is substrate concentrations, and k_{cat} and k_m are constants. In eq 2, $f(e) = e$ and $g(X, K) = \frac{k_{cat}X_S}{k_m + X_s}$.

When a metabolic system is subjected to any perturbation, eq 1 always leads to eq 3 [18].

$$1 = \frac{\Delta \log(f(e))}{\Delta \log(v)} + \frac{\Delta \log(g(X, K))}{\Delta \log(v)} = \rho_h + \rho_m \qquad \text{(eq 3)}$$

ρ_h is the ‘‘hierarchical regulation coefficient’’, quantifying the relative contribution of changes in active enzyme concentration to the regulation of the enzyme’s flux. ρ_m is the ‘‘metabolic regulation coefficient’’, quantifying the relative contribution of changes in the interaction of the enzyme with the rest of metabolism to the regulation of the enzyme’s flux [18-21].

Elucidating the regulation of hormonal crosstalk in root development

Hormonal crosstalk networks for plant root development can be constructed by iteratively combining modelling with experimental analysis [9]. Figure 1 shows the crosstalk network of auxin, ethylene and cytokinin via the interaction of PIN and PLS [10]. For this signalling system, change in the level or activity of one component (e.g., PLS protein, PLSp) may cause changes in other components (e.g. PIN1/PIN2 protein, auxin, ethylene and cytokinin).

--- Figure 1 here ---

Regulation analysis can be adapted to elucidate the regulation of hormonal crosstalk networks. Here, as a simple example, we analyse the regulation of auxin biosynthesis. In Figure 1, v_2 is auxin biosynthesis rate, which is described by eq 4 [9].

$$v_2 = k_2 + \frac{k_{2a}[ET]}{1+\frac{[CK]}{k_{2b}}}\frac{[PLS_P]}{k_{2c}+[PLS_p]} \qquad \text{(eq 4)}$$

Following the methodologies developed for regulation analysis [18-21], regulation coefficients for auxin biosynthesis can be derived (eq 5):

$$1 = \rho_b + \rho_{ET} + \rho_{CK} + \rho_{PLSp} \qquad \text{(eq 5)}$$

where $\rho_b = \frac{\Delta \log\left(\frac{k_2}{\frac{k_{2a}[ET]}{1+\frac{[CK]}{k_{2b}}}\frac{[PLS_P]}{k_{2c}+[PLS_p]}}\right)}{\Delta \log(v_2)}$ is the regulation coefficient of background auxin biosynethsis to auxin biosynthesis; $\rho_{ET} = \frac{\Delta \log(k_{2a}[ET])}{\Delta \log(v_2)}$ is the regulation coefficient of ethylene to auxin biosynthesis; $\rho_{CK} = -\frac{\Delta \log\left(1+\frac{[CK]}{k_{2b}}\right)}{\Delta \log(v_2)}$ is the regulation coefficient of cytokinin to auxin biosynthesis; $\rho_{PLSp} = \frac{\Delta \log\left(\frac{[PLS_P]}{k_{2c}+[PLS_p]}\right)}{\Delta \log(v_2)}$ is the regulation coefficient of PLSp to auxin biosynthesis.

We note that a regulation coefficient includes information at two levels: first, the relationship between a property and auxin biosynthesis; and second, how a specific perturbation (e.g, exogenous application of ethylene) has caused changes in both this property and auxin biosynthesis. For example, ρ_{PLSp} includes information on a) if others do not change, increasing PLSp increases auxin biosynthesis, as described by eq. 4; b) how a perturbation (e.g., exogenous application of ethylene) changes PLSp concentration and auxin biosynthesis.

When ethylene is exogenously applied, the rate of auxin biosynthesis increases [22,23]. However, PLSp concentration decreases [7,8]. Therefore, $\rho_{ET} > 0$ and $\rho_{PLSp} < 0$, implying that, when ethylene is exogenously applied, the increase in auxin biosynthesis rate is positively regulated by ethylene concentration, but negatively regulated by PLSp concentration. Following eq 4, decreasing PLSp concentration should decrease auxin biosynthesis rate if others do not change. However, when ethylene is exogenously applied, a decreased PLSp concentration actually corresponds to an increased auxin biosynthesis rate. Therefore, PLSp concentration negatively regulates auxin biosynthesis in this context. Due to the restriction of eq 5, quantification of any three regulation coefficients in eq 5 can determine the fourth regulation coefficient.

Other regulation coefficients in hormonal crosstalk network (Figure 1) can be derived based on the same principle. By quantifying the regulation coefficients, it is possible to quantify how each component regulates each flux or concentration in Figure 1, in a similar manner to the quantification of regulation in metabolic networks [18-21].

Elucidating the regulation of pollen tube growth

Hydrodynamics, cell wall and ion dynamics are important properties regulating pollen tube growth [11-17]. Therefore, pollen tube growth must be analysed as an integrated system [24-26].

Here, as a simple example, we show how regulation analysis can be used to analyse the regulation of cytosolic osmotic pressure by four major ions (Ca^{2+}, K^+, H^+, and Cl^-) in the pollen tube.

Cytosolic osmotic pressure, π_i , is related to four major ions by eq 6:

$$\pi_i = RT([Ca^{2+}]_i + [H^+]_i + [K^+]_i + [Cl^-]_i + [Osm]_i) \qquad \text{(eq 6)}$$

where R is gas constant, T is temperature. $[Ca^{2+}]_i$, $[H^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ are cytosolic concentrations of these ions. $[Osm]_i$ is the concentration of other cytosolic molecules that contribute osmolarity in cytosol.

Eq 6 always leads to eq 7 when pollen tube growth is subjected to any perturbation:

$$1 = \frac{RT\Delta[Ca^{2+}]_i}{\Delta\pi_i} + \frac{RT\Delta[H^+]_i}{\Delta\pi_i} + \frac{RT\Delta[K^+]_i}{\Delta\pi_i} + \frac{RT\Delta[Cl^-]_i}{\Delta\pi_i} + \frac{RT\Delta[Osm]_i}{\Delta\pi_i} \qquad \text{(eq 7)}$$

$$= \rho_{Ca^{2+}} + \rho_{H^+} + \rho_{K^+} + \rho_{Cl^-} + \rho_{Osm_i}$$

where $\rho_{Ca^{2+}}$, ρ_{H^+} , ρ_{K^+} , ρ_{Cl^-} and ρ_{Osm_i} are the regulation coefficients of Ca^{2+}, H^+, K^+, Cl^- and other molecules in cytosol to cytosolic osmotic pressure. For an oscillatory growth of the pollen tube, phase shifts may exist in the four ions [27,28]. Therefore, at any time, how Ca^{2+}, H^+, K^+, Cl^- regulates cytosolic osmotic pressure can be quantified by calculating their regulation coefficients. As cytosolic osmotic pressure is directly related to the hydrodynamics of pollen tube growth, it is possible to analyse how four ions regulate pollen tube growth via their regulation of hydrodynamics. Following the same principle, it should be possible to analyse comprehensively the regulation of hydrodynamics, cell wall and ion dynamics in relation to pollen tube growth.

Conclusion and future perspective

Signalling systems in plant cells involve complex interactions, in which change in one signalling component may cause changes in other signalling components. Thus, those components in a signalling system cannot change independently and a signalling system in plant cells must be analysed as an integrated system. In plant root development, hormones and related genes form a complex interacting network, and neither can change independently. Therefore, plant root development is regulated by a hormonal crosstalk [9,10]. In addition, hydrodynamics, cell wall and ion dynamics work together to regulate pollen tube growth [25,26]. For the signalling systems in both root development and pollen tube growth, regulation analysis, in which regulation coefficients reveals the regulation strength of each component, provides a powerful tool for analysing regulation in the two signalling systems.

The hormonal crosstalk network shown in Figure 1 includes three phytohormones: auxin, ethylene and cytokinin [10]. Other phytohormones such as gibberellin, abscisic acid and brassinosteroids are also important signals in the regulation of root development [2,5,6]. Although the effects of gibberellin and brassinosteroids on root development have been subjected to mathematical modelling studies [29,30], the networks describing their crosstalk with other hormones have not been constructed. One of the important future directions in hormonal crosstalk is to further integrate the hormonal crosstalk for other phytohormones and

genes [10]. Regulation of the constructed hormonal crosstalk networks can be further elucidated using regulation analysis, as described in this review.

Furthermore, manipulation of certain genes may change growth rate of the pollen tube. For example, knockout of Raba4d, a member of the Rab family of small GTPase proteins involved in vesicle transport, decreases pollen tube length [31]. When the roles of genes in pollen tube growth are investigated, a key challenge is to establish the relationships between the functions of genes and the properties of hydrodynamics, cell wall and ion dynamics. Once such relationships are established, regulation analysis can be applied to elucidate how the functions of genes regulate pollen tube growth.

In general, all biological networks such as metabolic, signalling and transcriptional networks in plant cells are dynamic [32]. For example, oscillations are the main features in both pollen tube growth [27] and stomatal guard cell behaviour [33]. In order to analyse how the dynamics of plant cells is regulated, time-dependent regulation analysis [34] can be adapted to develop useful modelling tools.

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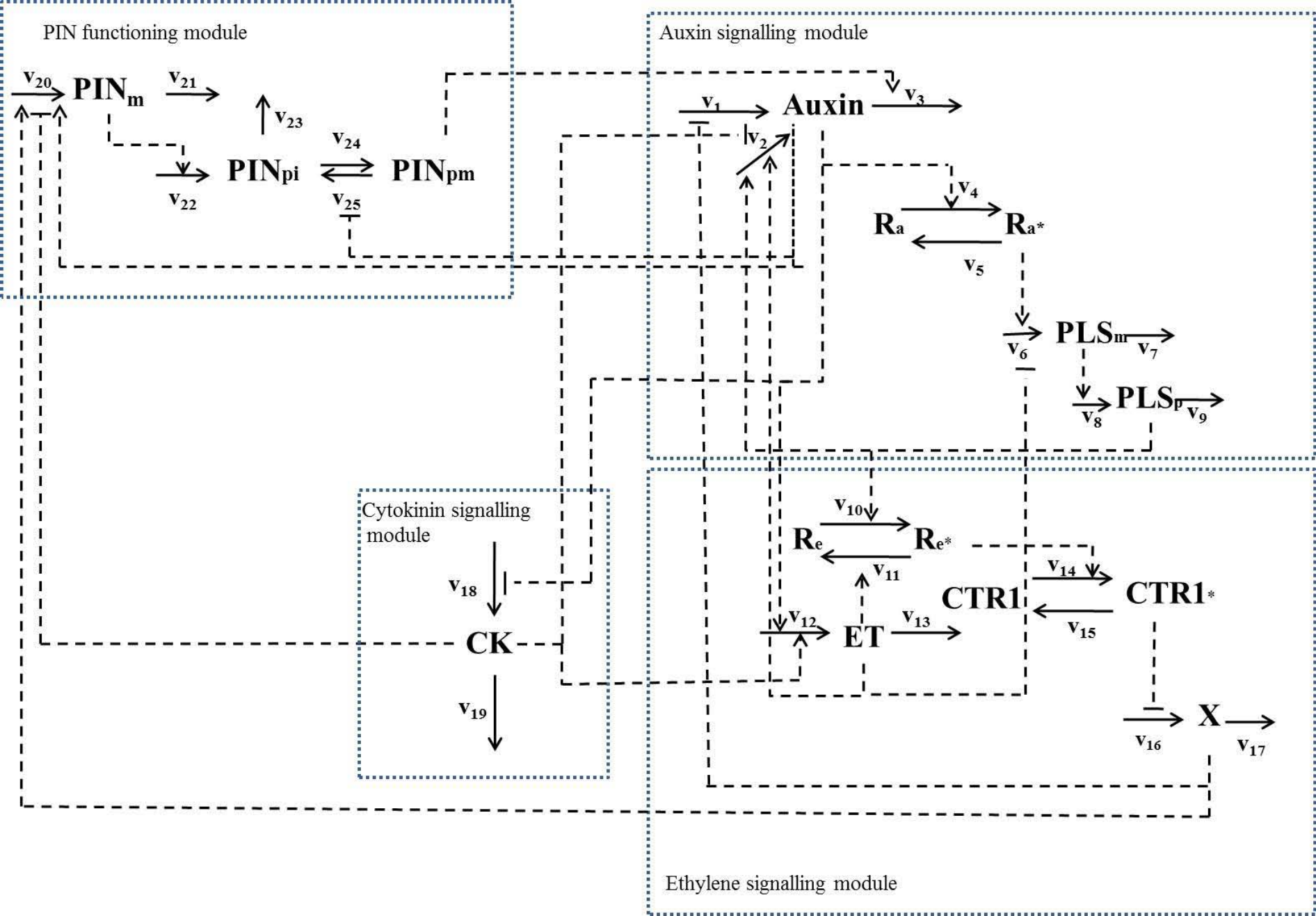
Figure legend

Figure 1. Network for the interaction of PIN and PLS and hormonal crosstalk in the situation in which cytokinin decreases endogenous auxin concentration. The reaction rates are: v1: total auxin influx from all neighboring; v2: auxin biosynthesis rate in the cell; v3: total auxin efflux from the cell; v4: rate for conversion of the inactive form of the auxin receptor to its active form; v5: rate for conversion of the active form of the auxin receptor to its inactive form; v6: transcription rate of the POLARIS (PLS)gene; v7: decay rate of PLS mRNA; v8: translation rate of the PLS protein; v9: decay rate of PLS protein; v10: rate for conversion of the inactive form of the ethylene receptor to its active form by PLS protein (PLSp); v11: rate for conversion of the active form of ethylene receptor to its inactive form; v12: ethylene biosynthesis rate; v13: rate for removal of ethylene; v14: rate for conversion of the inactive form of the CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) protein to its active form; v15: rate for conversion of the active form of CTR1 protein to its inactive form; v16: rate for activation of the ethylene signalling response; v17: rate for removal of the unknown ethylene signalling component, X; v18: rate for cytokinin biosynthesis; v19: rate for removal of cytokinin; v20: transcription rate of the PIN gene; v21: rare for the decay of PIN mRNA; v22: translation rate of PIN protein; v23: rate for decay of PIN protein in cytosol; v24: rate for transport of PIN protein from cytosol to plasma membrane; v25: rate for internalisation of PIN protein. When exogenous hormones are applied: v26: rate for uptake of IAA when exogenous IAA is applied; v27: rate for uptake of ACC when exogenous ACC is applied; v28: rate for uptake of cytokinin when exogenous cytokinin is applied. (Figure is reproduced with permission from Liu et al., 2013 [10] and figure legend is modified).


PIN functioning module
Auxin signalling module
Cytokinin signalling module
Ethylene signalling module
v20
PINm
v21
v23
v24
PINpi
PINpm
v22
v25
v1
Auxin
v3
v2
v4
Ra
Ra*
v5
v6
PLSm
v7
v8
PLSp
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