framework (*27*), *Sarcosuchus* and its closest relatives are understood as basal neosuchians, allied with but outside Crocodylia and the radiation that gave rise to all living crocodylians.

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17. The holotypic skull of *S. imperator* in the collections of the Musée National du Niger (MNN) has a length of 160 cm (*2*) and is the largest verified skull length on record (the same reference reported a 170-cm skull, but there are no figures of this specimen).
18. Regressions of body length (y) against head length (x) for *G. gangeticus* ($y = -69.369 + 7.4x$, $r^2 = 0.972$) and *C. porosus* ($y = -20.224 + 7.717x$, $r^2 = 0.98$) yield total body length estimates of 11.15 m and 12.15 m, respectively, for a skull length of 160 cm. Mean total body length equals 11.65 m (38 feet, 3 inches) (Fig. 4B). Data for *G. gangeticus* ($n = 17$) include measurements taken by P. Sereno from captive bred gharials in the Kukrail Picnic Center and Katerniaghat National Reserve in northern India and available measurements for individuals greater than 1.5 m long (*11*). Data for *C. porosus* ($n = 28$) come from wild individuals in northern Australia (A. Britton) and from available measurements for individuals greater than 1.5 m long (*11*) (excluding one suspicious record with body length of 4.91 m) (for measurement data, see supplementary material) (*29*).
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ARR1, a Transcription Factor for Genes Immediately Responsive to Cytokinins

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Cytokinins are a class of phytohormones involved in various physiological events of plants. The *Arabidopsis* sensor histidine kinase CRE1 was recently reported to be a cytokinin receptor. We used a steroid-inducible system to show that the transcription factor–type response regulator ARR1 directs transcriptional activation of the *ARR6* gene, which responds to cytokinins without de novo protein synthesis. This fact, together with characteristics of *ARR1*-overexpressing plants and *arr1* mutant plants, indicates that the phosphorelay to ARR1, probably from CRE1, constitutes an intracellular signal transduction occurring immediately after cytokinin perception.

Cytokinins induce a variety of physiological events, including cell division, chloroplast development, and shoot formation (*1*, *2*). These cytokinin responses in *Arabidopsis* are at least partly triggered through the recognition of cytokinins by the sensor protein CRE1, which is a member of the protein histidine kinase family (*3*, *4*). In bacteria, histidine kinases participate in His-Asp phosphorelays, which respond to environmental stimuli, usually in association with cytoplasmic response regulators, the majority of which are transcription factors (*5*). If the His-Asp phosphorelay is also the case in plants, an intracellular signal transduction pathway starting from the CRE1 sensor may involve response regulators. The *Arabidopsis* genome codes for 22 response regulators (ARRs), 12 of which contain a Myb-like DNA binding domain called ARRM (type B) (*6–9*). The remainder (type A) possess no apparent functional unit other than a signal receiver domain containing two aspartate and one lysine residues (DDK) at invariant positions, and their genes are transcriptionally induced by cytokinins without de novo protein synthesis (*7*, *9*, *10*). The type B members, ARR1 and ARR2, bind DNA in a sequence-specific manner and work as transcriptional activators (*11*, *12*).

We analyzed the morphological characteristics of transgenic plants carrying *35S::ARR1* and *35S::ARR1ΔDDK* genes (*13*), in which the full-length ARR1 and its truncated version missing

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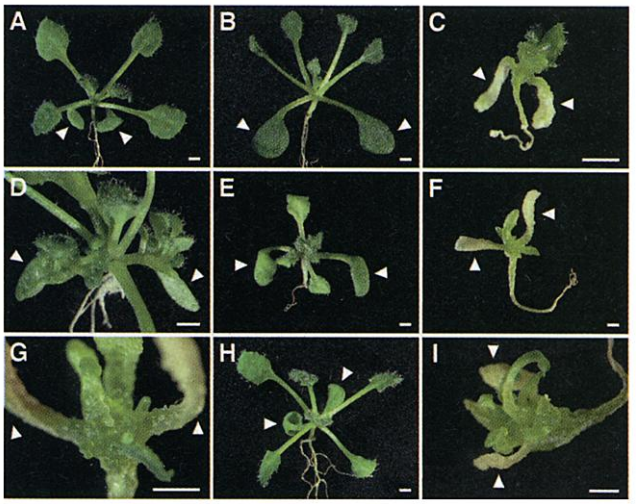

Fig. 1. Phenotypes of transgenic plants. (**A** and **E**) Wild-type plants grown without and with 1 μM BA, respectively. (**B** and **F**) *35S::ARR1* plants grown without and with 1 μM BA, respectively. (**G**) An enlargement of (F). (**C** and **D**) *35S::ARR1ΔDDK* plants without BA. (**H** and **I**) *35S::ARR1ΔDDK::GR* plants without and with 0.1 μM DEX, respectively. Arrowheads indicate cotyledons. Scale bars, 1 mm.

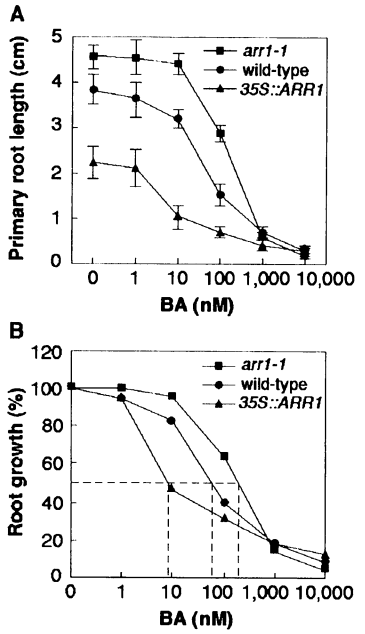

Fig. 2. Root growth of wild-type (circles), *35S::ARR1* (triangles), and *arr1-1* (squares) plants in the presence of exogenous cytokinin. Seeds were germinated and grown on vertical agar medium containing various concentration of BA. (**A**) The primary root length of 8-day-old seedlings was measured, and the average and standard deviations from at least 15 individuals for each condition were calculated and plotted against the BA concentration. (**B**) Root growth is expressed relative to the mean root length of respective plants grown without BA. Dashed lines indicate the BA concentrations causing IC_{50}.

the NH_2-terminal signal receiver domain, respectively, were directed by the cauliflower mosaic virus 35*S* promoter. On the one hand, transgenic *35S::ARR1* plants showed no phenotypic change under normal growth conditions, except for hypertrophic cotyledons, longer cotyledonary petioles, and shorter roots than wild-type plants (Fig. 1, A and B, and Fig. 2A). On the other hand, *35S::ARR1ΔDDK* plants did show a phenotypic change under normal growth conditions, forming ectopic shoots on the adaxial surface of their cotyledons (Fig. 1D). In lines expressing the transgene at relatively high levels, plant growth was occasionally inhibited, with concomitant disordered cell proliferation around the shoot apex (Fig. 1C). Because the transactivating function of ARR1 was masked by its signal receiver domain in transient expression experiments (*12*), the phenotypes generated by *35S::ARR1ΔDDK* likely resulted from the constitutive transactivating function of ARR1ΔDDK, independent of a signal from an upstream component. To test whether the cytokinin signal regulates ARR1 through its signal receiver domain, we treated *35S::ARR1* plants with exogenous 6–benzylaminopurine (BA) at various concentrations and compared their morphologies with those of nontreated *35S::ARR1ΔDDK* plants. Transgenic *35S::ARR1* plants grown in the presence of 1 μM BA showed growth inhibition concomitant with disordered cell proliferation around the shoot apex (Fig. 1, F and G). This phenotype was similar to a severe phenotype seen in nontreated *35S::ARR1ΔDDK* plants (Fig. 1C). Other kinds of cytokinins (kinetin, trans-zeatin, and isopentenyladenine) had similar effects on *35S::ARR1* plants, whereas a different phytohormone, auxin (3-indoleacetic acid), did not (*14*). These results suggest that the signal receiver domain of ARR1 suppresses the function of ARR1 in the absence of cytokinins and that a cytokinin signal releases the suppression. Treatment of wild-type plants with BA caused growth inhibition but not the disordered cell proliferation that was observed in *35S::ARR1* plants. (Fig. 1E). This difference might imply that ectopic overexpression of *ARR1* disturbs homeostasis in cytokinin responses.

Another finding from morphological analyses is that plants overexpressing *ARR1* appear to be more sensitive to cytokinins than are wild-type plants (Fig. 1, E and F). To investigate the relationship between sensitivity to cytokinins and level of *ARR1* expression, we examined wild-type, *35S::ARR1*, and *arr1-1* mutant plants by two different procedures: root-elongation and callus-formation assays. The *arr1-1* mutant obtained by screening transferred DNA (T-DNA)–tagged lines (*15*) contains a T-DNA insertion within the first intron of the *ARR1* gene and produces no mature *ARR1* mRNA (see below). Apparent phenotypic alterations were not detected in *arr1-1* plants, except for longer roots than in wild-type plants under normal growth conditions (Fig. 2A). In the root-elongation assay, we grew seedlings on agar media containing various concentrations of BA. In the absence of BA, *35S::ARR1* and *arr1-1* plants had shorter and longer roots than did wild-type plants, respectively. BA inhibited root growth in all the plants at concentrations of 10 nM and higher (Fig. 2). However, roots of *35S::ARR1* and *arr1-1* plants were more and less sensitive, respectively, to BA than were those of wild-type plants (see IC_{50}, the concentration of BA needed to cause 50% inhibition of root growth, shown in Fig. 2B). In the callus-formation assay, we tested the response (green callus formation) of tissue-cultured explants to varying concentrations of the cytokinin kinetin and to the auxin 2,4-dichlorophenoxyacetic acid (2,4-D), using the procedure described by Kubo and Kakimoto (*16*). Like wild-type explants, *35S::ARR1* and *arr1-1* explants responded to increasing levels of kinetin with rapid proliferation and greening, and in the case of *35S::ARR1* explants, occasionally with shoot formation (Fig. 3). However, the sensitivity of *35S::ARR1* and *arr1-1* explants to kinetin was higher and lower, respectively, than that of wild-type explants. These results show that the level of *ARR1* expression correlates with sensitivity to cytokinin, which supports the view that ARR1 is involved in the cytokinin signal transduction pathway.

To gain insight into how ARR1 contributes to cytokinin signal transduction at the molecular level, we examined transcription levels of a cytokinin-responsive marker gene in *35S::ARR1* and *arr1-1* plants, with or without BA treatment, by Northern blot analysis (*17*). The marker gene we chose was *ARR6* (*18*), a type A response regulator gene, because its expression is rapidly induced by cytokinins without de novo protein synthesis (*7*, *10*). As in wild-type plants, *ARR6* responded rapidly to BA in both *35S::ARR1* and *arr1-1* plants, even in the presence of cycloheximide (CHX), a protein synthesis inhibitor (Fig. 4A). However, the transcription levels induced by the BA treatment of *35S::ARR1* and *arr1-1* plants were higher and lower, respectively, than in wild-type plants. This induction pattern, together with the appearance of an ARR1 recognition sequence (5′ -AGATT-3′) (*12*) three times in the 200–base pair region upstream from the *ARR6* translation start site, suggests that the transactivation of *ARR6* is targeted by ARR1. Consistent with this, *35S::ARR1ΔDDK* plants showing severe phenotypes expressed *ARR6* at much higher levels than did wild-type plants

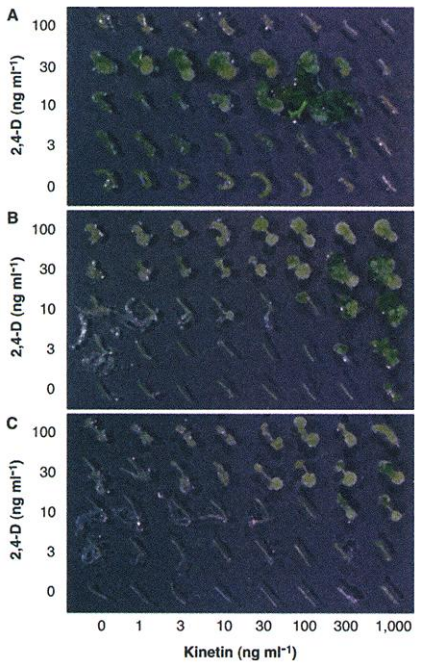

Fig. 3. Callus growth of *35S::ARR1*, wild-type, and *arr1-1* explants exposed to different cytokinin and auxin concentrations. Hypocotyl segments were excised and cultured on MS media containing different levels of kinetin and 2,4-D. After 21 days in culture, the induced calli were arranged and photographed. **(A)** *35S::ARR1* explants, **(B)** wild-type explants, and **(C)** *arr1-1* explants.

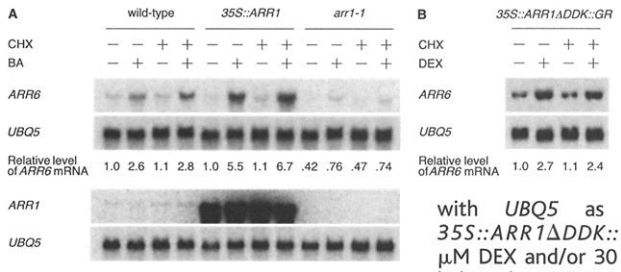

Fig. 4. Northern blot analysis for *ARR6* transcription. **(A)** Wild-type, *35S::ARR1*, and *arr1-1* plants were treated with 5 μM BA and/or 30 μM CHX for 30 min and then subjected to Northern blot analysis for *ARR6* and *ARR1*, with *UBQ5* as a control. **(B)** Transgenic *35S::ARR1ΔDDK::GR* plants were treated with 30 μM DEX and/or 30 μM CHX for 1 hour. Numerals below the *UBQ5* signals indicate the level of *ARR6* mRNA that was quantified by measuring the radioactivity of the signal band with a Fujix BA100 Bio-Image analyzer, divided by the radioactivity of the *UBQ5* signal band with the same RNA sample for normalization and presented as the relative value to that for wild-type plants (A) or *35S::ARR1ΔDDK::GR* plants (B) without chemical treatment.

under normal growth conditions (*14*). Direct transactivation of *ARR6* by ARR1 was shown with transgenic plants carrying a chimeric *35S::ARR1ΔDDK::GR* gene, whose translation product was expected to become activated by glucocorticoid without de novo protein synthesis (*19*). Such steroid-inducible systems using the hormone-binding domain of the glucocorticoid receptor (GR) have been used as powerful tools to identify direct target genes for various transcription factors (*20–22*). Transgenic *35S::ARR1ΔDDK::GR* plants were indistinguishable from wild-type plants under normal growth conditions (Fig. 1H), but the addition of a synthetic glucocorticoid, dexamethasone (DEX), caused graduated morphological changes, which were dependent on the DEX concentration. A plant treated with 0.1 μM DEX showed growth inhibition concomitant with disordered cell proliferation around the shoot apex (Fig. 1I), which was similar to a severe phenotype in *35S::ARR1ΔDDK* plants (Fig. 1C). These results indicate that ARR1 function can be modulated by DEX instead of by cytokinins in this system. Treatment of *35S::ARR1ΔDDK::GR* plants with DEX for 1 hour enhanced *ARR6* transcription, even in the presence of CHX (Fig. 4B) (*17*). This provides in vivo evidence for direct interaction of ARR1ΔDDK::GR with the *ARR6* gene. This in vivo evidence, taken together with the higher and lower *ARR6* transcription levels in BA-treated *35S::ARR1* and *arr1-1* plants, respectively, versus wild-type plants, lead us to conclude that ARR1 directly activates *ARR6* transcription in response to cytokinins.

In *Arabidopsis*, cytokinin receptors, ethylene receptors, and, possibly, osmosensors are histidine kinases (*3*, *4*, *23–25*). However, their cognate response regulators, working as transcription factors, have never been identified. Here we present evidence that ARR1 mediates a cytokinin signal, probably through its NH_2-terminal signal receiver domain, and transactivates *ARR6*, which is immediately responsive to cytokinins. A paralogous response regulator, ARR2, shows almost identical characteristics to ARR1 (*12*), suggesting a functional overlap. Residual cytokinin responses observed with the *arr1-1* mutant may have been provided by ARR2. In addition to *ARR6*, other type A member genes, including *ARR4*, *ARR5*, *ARR7*, *ARR8*, and *ARR9*, were also activated by DEX at various levels in *35S::ARR1ΔDDK::GR* plants (*14*), suggesting that all the immediate cytokinin-responsive genes belonging to this group are directly activated by ARR1. Also, other cytokinin-responsive genes whose promoter regions contain the ARR1 recognition sequences are possibly transactivated by ARR1. A screening for ARR1 target genes using transgenic *35S::ARR1ΔDDK::GR* plants will shed light on the whole view of the early cytokinin signal transduction pathway. We conclude that ARR1 is a principal transcription factor–type response regulator that is involved in an early step of cytokinin signal transduction, possibly as a partner of the sensor histidine kinase CRE1.

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15. We obtained the T-DNA insertion mutant *arr1-1* (Columbia ecotype) using the screening system developed at the Kazusa DNA Research Institute. The principles of the screening method were as described (*27*). Synthetic primers 5′-ACCCAGAATTTGAGCTCAAAGC-3′, 5′-GTGCAATCCCTTCCTGTATATC-3′, and 5′-CAGCAGCAACAAACTGCTGATG-3′ corresponded to the *ARR1* gene; and the primers 5′-AAGAAAATGCCGATACTTCATTGGC-3′ and 5′-TTCCCTTAATTCTCCGCTCATGATC-3′ corresponded to the T-DNA left and right borders, respectively, of the pGTAC–LUS vector. Six pairs of primers, each consisting of an *ARR1*-specific primer and a T-DNA–specific primer, were used for polymerase chain reactions. The position of the T-DNA insert was determined by sequencing the junction between the T-DNA and the genome.
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