A Universal Identifier for Computational Results

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July 14, 2011

35:00

Prof. David Donoho

Credibility Crisis in Scientific Communication

Jon Claerbout, 1990 (paraphrase Donoho and Buckheit, 1995)

> *An article about computational science in a scientific publication is not the scholarship itself, it is merely advertising of the scholarship. The actual scholarship is the complete software development environment and the complete set of instructions which generated the figures.*

Advances 1995 – 2011 ?

- Only RR fans attempt follow RR practices
- No-one expects or enforces reproducibility
- No uniform standards of reproducibility $\longrightarrow$ no established user base

- "Most published scientific research is false".
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Why?

Lesson learned: For RR solution to become standard, it must be –

- Technologically and legally realistic
- Easy for everyone to adopt today
- Offer real benefits for everyone

Everyone: researchers, authors, readers, publishers

This talk is about an RR solution that was designed to become standard.
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Microarray analysis of replicative senescence

Dawne N. Shelton*, Edwin Chang†, Peter S. Whittier*, Donghee Choi‡ and Walter D. Funk*

Background: Limited replicative capacity is a defining characteristic of most normal human cells and culminates in senescence, an arrested state in which cells remain viable but display an altered pattern of gene and protein expression. To survey widely the alterations in gene expression, we have developed a DNA microarray analysis system that contains genes previously reported to be involved in aging, as well as those involved in many of the major biochemical signaling pathways.

Results: Senescence-associated gene expression was assessed in three cell types: dermal fibroblasts, retinal pigment epithelial cells, and vascular endothelial cells. Fibroblasts demonstrated a strong inflammatory-type response, but shared limited overlap in senescent gene expression patterns with the other two cell types. The characteristics of the senescence response were highly cell-type specific. A comparison of early- and late-passage cells stimulated with serum showed specific deficits in the early and mid G1 response of senescent cells. Several genes that are constitutively overexpressed in senescent fibroblasts are regulated during the cell cycle in early-passage cells, suggesting that senescent cells are locked in an activated state that mimics the early remodeling phase of wound repair.

Conclusions: Replicative senescence triggers mRNA expression patterns that vary widely and cell lineage strongly influences these patterns. In fibroblasts, the senescent state mimics inflammatory wound repair processes and, as such, senescent cells may contribute to chronic wound pathologies.

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Received: **16 March 1999**
Revised: **2 July 1999**
Accepted: **15 July 1999**

Published: **23 August 1999**

Current Biology 1999, 9:939–945
http://biomednet.com/elecref/0960982200900939

0960-9822/99/$ – see front matter

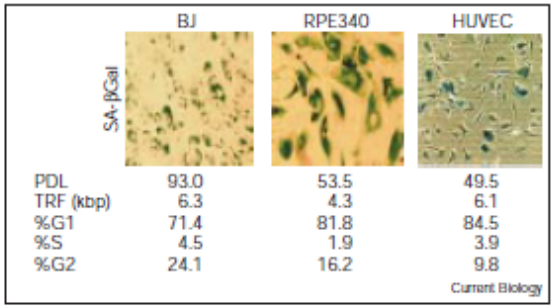
Background

Historically, cellular senescence has been defined as the terminal phase of passaged primary human cell populations, a response more accurately defined as replicative senescence. It is now recognized, however, that a similar phenotype can be achieved in both normal and transformed cells by a variety of challenges, such as oxidative stress [1,2], radiation [3], activated oncoproteins and kinases [4–6], cyclin-dependent kinase (CDK) inhibitors [7] and others [8], leading to the concept that cellular senescence represents a fate choice that can be influenced by both pro- and anti-mitogenic stimuli. Senescent cells are not stimulated to divide by serum or passage in culture, and senescence invokes a specific cell-cycle understand the baseline changes in gene expression at senescence, we have used cDNA microarrays to study senescence-induced gene expression patterns in different cell types. The cell types examined here — dermal fibroblasts, retinal pigment epithelial cells and vascular endothelial cells — represent biologically diverse lineages that senesce with similar gross phenotypes. The invoked gene expression patterns differ greatly in a cell-type specific manner, however, suggesting that many of the observed alterations result from collateral activation of pathways that are set by cell lineage.

Results

Expression profiles of cell strains at replicative senescence

Figure 1



Analyses of senescent primary human cell lines. Photomicrographs are of senescent cultures stained for senescence-associated β-galactosidase (SA-βGal) [10]. Population doubling levels (PDLs) for senescent cultures are indicated, as well as telomere length (mean TRF in kilobase pairs (kbp)), and cell-cycle stage distribution as determined by multicycle FACS analysis.

RPE340 [14] and HUVEC (E.C., unpublished observations) can be immortalized by the expression of telomerase from an hTERT transgene. By these criteria, all three strains of cells undergo a similar arrest process — replicative senescence — initiated by a common effector, the critical shortening of telomeres.

Genes showing at least a 2.5-fold differential in expression in senescent and early-passage cells in multiple independent experiments are listed in Figure 2. Compared with quiescent early-passage cells, BJ fibroblasts at senescence have higher levels of the matrix-regulating proteins stromelysin-1 and stromelysin-2, plasminogen activator inhibitors PAI-1 and PAI-2, and urokinase plasminogen activator (uPA) as well as the inflammatory regulators monocyte chemotactic protein-1 (MCP-1), Gro-α, and interleukin-15 (IL-15). Conversely, senescent fibroblasts significantly underexpressed mRNAs for prostaglandin-1 synthase, elastin, stromelysin-3 and other proteins.

In high-serum conditions, genes that participate in cell division (for example those for cyclin A, cyclin B1, Cdc20, Cdc2, thymidine kinase and thymidylate synthase) are clearly expressed at higher levels in early-passage fibroblasts. Under these conditions, genes for secreted collagens Iα1 and IIIα1, elastin and cytokeratin II type 7 are also more highly expressed in early passage cells.

(Gas1) and growth-arrest and DNA-damage inducible protein 153 (GADD153). In addition, levels of mRNA for insulin-like growth factor binding proteins 2 and 5 (IGF-BP2, IGF-BP5), stanniocalcin and cathepsin O were elevated, and levels of peptidyl-α amidating monooxygenase (PAM), a prohormone-converting enzyme [16], remained high regardless of serum conditions.

The expression patterns observed in the other two cell types differed significantly from that of the dermal fibroblasts. In RPE340 cells, the differences in expression of mRNA for IGF-BP2, in both high and low serum, and a notable inability of the senescent cells to express a series of collagens in low-serum conditions were most obvious. Follistatin, a potential activin antagonist protein [17], was significantly expressed in senescent RPE340s whereas senescence repressed the expression of prostaglandin D synthase, a potential retinoid-binding protein [18], and cellular retinol-binding protein-1. Unlike BJ cells, senescence in RPE340s does not result in a significant increase in the expression of inflammatory chemokines or cytokines.

HUVEC cells display a dramatically different pattern of gene expression, in which many of the markers of senescence in fibroblasts are expressed in an inverse fashion. Inflammatory and immune-response genes such as those for IL-15 and Toll protein are all repressed in senescent HUVECs at low serum, as are the matrix proteases. Senescence in HUVECs induces the expression of IGF-BP5, neurofilament subunit L, transforming growth factor-β2 (TGFβ2) and adenosine A2A receptor, regardless of serum conditions. In high-serum conditions, ICAM-1 and GADD153 are overexpressed at senescence, as in fibroblasts.

To assess the extent that senescence-associated patterns of gene expression are preserved among cell strains of similar type, we examined two additional dermal fibroblast lines. C4 and MA were derived from the hand and ankle respectively of the same donor and are thus genetically identical. In general, patterns of gene expression in these two lines varied from that of BJ fibroblasts, though some shared trends are apparent. The expression of matrix proteases and inflammatory chemokines and cytokines at senescence is common to all the dermal fibroblast lines, though the set of expressed markers varies (Table 1a). A comparison of the MA and C4 strains suggests that the magnitude of the response at senescence and the particu-

Figure 2

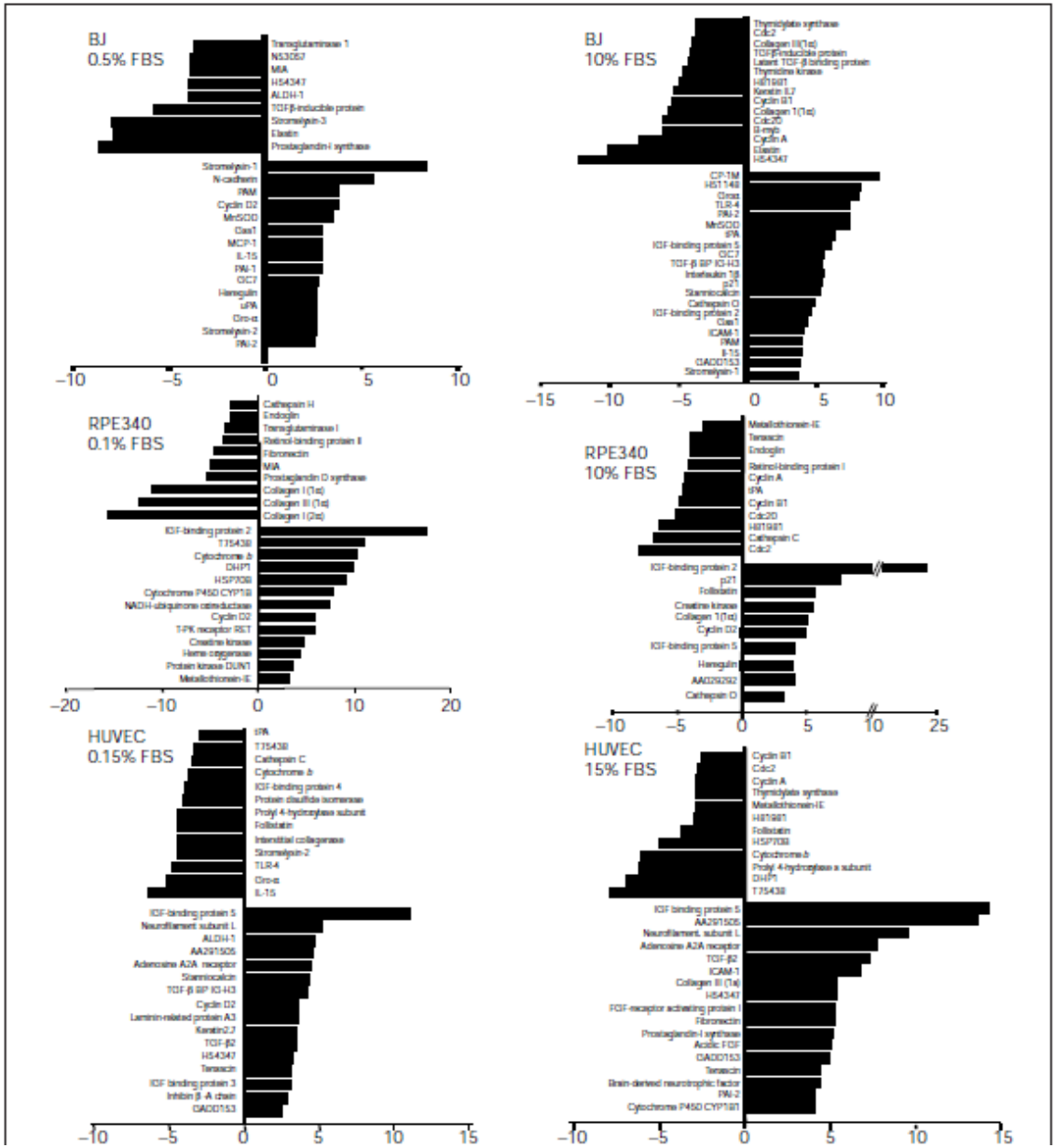

BJ
0.5% FBS
Transglutaminase 1
N53057
MIA
H54347
ALDH-1
TGFβ-inducible protein
Stromelysin-3
Elastin
Prostaglandin-I synthase
Stromelysin-1
N-cadherin
PAI-1
Cyclin D2
MnSOD
Cox7
MCP-1
IL-1S
PAI-1
GIC7
Heregulin
uPA
Gro-α
Stromelysin-2
PAI-2
−10 −5 0 5 10
BJ
10% FBS
Thymidylate synthase
Cdc2
Collagen III(1α)
TGFβ-inducible protein
Latent TGF-β binding protein
Thymidine kinase
H87/88
Keratin 8.7
Cyclin B1
Collagen I(1α)
Cdc25
B-myb
Cyclin A
Elastin
H54347
CIP-1M
H51148
Gros
TLR-4
PAI-2
MnSOD
tPA
IGF-binding protein 5
GIC7
TGF-β BP IG-H3
Interleukin 18
p21
Stanniocalcin
Cathepsin D
IGF-binding protein 2
Cox7
ICAM-1
PAM
IL-1S
GADD153
Stromelysin-1
−15 −10 −5 0 5 10
RPE340
0.1% FBS
Cathepsin H
Endoglin
Transglutaminase I
Retinol-binding protein II
Fibronectin
MIA
Prostaglandin D synthase
Collagen I (1α)
Collagen III (1α)
Collagen I (2α)
IGF-binding protein 2
T75438
Cytochrome b
DHFR
HSP70B
Cytochrome P450 CYP1B
NADH-ubiquinone oxidoreductase
Cyclin D2
T-PK receptor RET
Creatine kinase
Heme oxygenase
Protein kinase DUN1
Metallothionein-IE
−20 −10 0 10 20
RPE340
10% FBS
Metallothionein-IE
Tenascin
Endoglin
Retinol-binding protein I
Cyclin A
tPA
Cyclin B1
Cdc20
H87/88
Cathepsin C
Cdc2
IGF-binding protein 2
p21
Follistatin
Creatine kinase
Collagen I(1α)
Cyclin D2
IGF-binding protein 5
Heregulin
AA029292
Cathepsin D
−10 −5 0 5 10 25
HUVEC
0.15% FBS
tPA
T75438
Cathepsin C
Cytochrome b
IGF-binding protein 4
Protein disulfide isomerase
Prolyl 4-hydroxylase subunit
Follistatin
Interstitial collagenase
Stromelysin-2
TLR-4
Gro-α
IL-1S
IGF-binding protein 5
Neurofilament subunit L
ALDH-1
AA291505
Adenosine A2A receptor
Stanniocalcin
TGF-β BP IG-H3
Cyclin D2
Laminin-related protein A3
Keratin2.7
TGF-β2
H54347
Tenascin
IGF binding protein 3
Inhibin β -A chain
GADD153
−10 −5 0 5 10 15
HUVEC
15% FBS
Cyclin B1
Cdc2
Cyclin A
Thymidylate synthase
Metallothionein-IE
H87/88
Follistatin
HSP70B
Cytochrome b
Prolyl 4-hydroxylase α subunit
DHFR
T75438
IGF binding protein 5
AA291505
Neurofilament, subunit L
Adenosine A2A receptor
TGF-β2
ICAM-1
Collagen III (1α)
H54347
FGF-receptor activating protein 1
Fibronectin
Prostaglandin-I synthase
Acidic FGF
GADD153
Tenascin
Brain-derived neurotrophic factor
PAI-2
Cytochrome P450 CYP1B1
−10 −5 0 5 10 15

Table 1

(a) Pattern of gene expression in dermal fibroblasts at senescence.

	BJ		C4		MA	
%FBS	0.5	10	0.5	10	0.5	10
Stromelysin-1	8.2	4.1	1.0	22.0	–1.5	1.1
Stromelysin-2	2.6	2.0	1.6	5.9	1.9	1.6
Collagenase	2.0	1.7	–1.4	7.6	2.6	1.5
tPA	1.7	6.3	5.5	1.9	2.5	8.1
uPA	2.7	3.4	1.7	4.8	–1.2	1.2
MCP-1	2.9	9.0	2.7	4.3	2.5	3.4
Gro-α	2.8	6.6	1.6	5.8	2.5	1.2
IL-15	2.9	4.1	2.5	9.7	4.0	2.6
IL-1β	1.7	5.6	–1.2	3.1	1.3	1.2
Cathepsin O	2.9	4.8	4.2	3.9	2.2	4.2
Elastin	–8.4	–5.5	–4.5	–4.5	–8.3	–13.9
MnSOD	3.4	7.4	2.9	2.6	3.8	1.6
Tir-4	2.0	7.7	1.5	7.3	1.9	1.6
ICAM-1	–1.2	4.8	1.2	3.7	5.4	3.7

(b) Pattern of gene expression in retinal pigment epithelial cells at senescence.

	RPE340		RPE338		RPE341	
%FBS	0.1	10	0.1	10	0.1	10
IGF-binding protein-2	15.3	22.0	6.0	6.9	1.8	4.1
Collagen Iα2	–16.0	1.1	–10.6	–3.6	–58.0	–18.6
Collagen Iα1	–9.5	3.4	1.9	1.4	–26.5	–8.2
Collagen IIIα1	–9.8	1.4	1.9	3.3	–16.5	–4.0
Keratin I,18	1.4	–2.6	–3.8	–2.6	–8.1	–10.6
Keratin II,7	–1.0	–2.4	–18.5	–23.7	–24.8	–23.2
Transglutaminase 1	–3.5	–4.0	1.8	–1.9	–5.2	–3.0
Creatine kinase	3.6	7.3	3.9	2.3	1.6	2.8
Follistatin	–1.6	5.8	4.7	6.0	1.5	3.1
Cathepsin O	–2.1	3.0	2.5	2.7	24.3	11.7
Ceruloplasmin	1.4	1.7	1.0	3.7	14.6	7.0
Retinol-binding protein-1	–3.1	–3.2	1.2	1.2	–3.2	–4.4
Stanniocalcin	1.2	1.3	5.6	9.9	2.3	5.7
Thrombospondin 2	1.2	–3.5	–7.0	–3.3	–10.7	–16.8

RNA was prepared from three independent **(a)** dermal fibroblast or **(b)** RPE strains maintained in high or low serum. Positive numbers indicate fold-overexpression in senescent cultures; negative numbers indicate fold-overexpression in early passage cultures. See text and Supplementary material for abbreviations.

Time course of the response to serum stimulation

We compared the responses of early- and late-passage BJ fibroblasts to serum stimulation and a complete listing of responsive genes is provided as Supplementary material. In early-passage cells, late G1/early S-phase markers, such whereas in senescent cultures the response was attenuated (Figure 3a). Similarly, mRNAs for IκBα, tristetraproline (TTP), JunB and early growth response protein 1 (EGR-1) are all induced within 1 hour of stimulation in early-passage cells, whereas they are only weakly induced in senescent fibroblasts. The induction of mRNAs for connective tissue growth factor (CTGF) and mitogen-activated protein (MAP) kinase phosphatase 1 appeared unperturbed in senescent cells, indicating that substantial aspects of the early response to serum are maintained. Conversely, there was a pronounced induction of Id-2 in senescent cultures.

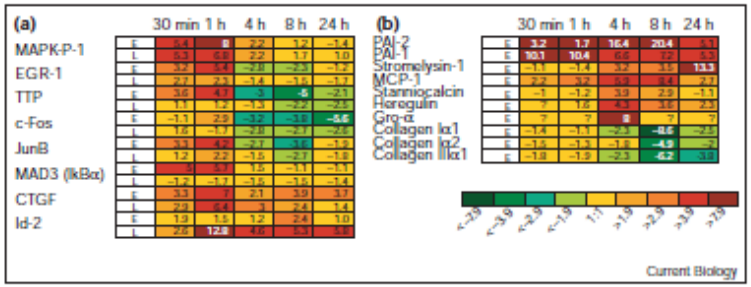
Several genes that are serum responsive in early-passage fibroblasts are constitutively upregulated in senescent cells. In early-passage cells, mRNAs for stromelysin-1, MCP-1, PAI-1, PAI-2 and others are induced by serum (Figure 3b). Conversely, collagen transcript levels decrease substantially following serum addition. The expression of many of the serum-inducible genes peaks near the 8 hour time point, at which time the cells would be in transit through the G1/S boundary.

Discussion

Senescence-associated gene expression

All three cell strains reported here undergo an arrest characterized by a distinctive cell-cycle distribution, changes in cell morphology and the shortening of telomeres. Senescent cells have a cell-cycle distribution that is clearly different from that of quiescent cells, as a substantial population of cells with G2 DNA content persists [9] (Figure 2). In high-serum conditions, this arrest includes expression of the CDK inhibitor p21, and growth arrest specific protein (Gas1) in BJ fibroblasts and RPE340 strains, but not in HUVECs. The overexpression of cyclin D2 was observed in all three cell strains and may reflect a common cell-cycle braking mechanism [19,20]. Interestingly, in none of the cell strains examined did we observe an upregulation of mRNA for the CDK inhibitor p16, as reported for other cell strains [21,22], and RT–PCR analysis of mRNA from senescent BJ fibroblasts confirmed the lack of induction of mRNA for p16 (data not shown). The induction of p21 at senescence has been reported to be transient in some cell lines, whereas the induction of p16 can occur after prolonged arrest [23], and this may also account for our observed results with different cell lines. The data presented here strongly suggest that even with a common initiating signal — shortened telomeres — the ensuing arrest may be triggered by dif-

Figure 3



Time course of serum stimulation. **(a)** Early passage (E: PD30) or late passage (L: PD89) BJ cultures were held in 0.5% serum for 2 days, then stimulated with 10% FBS. RNA levels from cultures at the indicated time points (Cy5 channel) were compared with the uninduced starting culture (Cy3 channel). Positive values indicate higher expression in induced cells; negative values indicate lower expression in induced cells. Question marks indicate that there was insufficient signal for detection. A complete listing of serum-responsive genes from this analysis is provided in Supplementary material. **(b)** The serum-responsiveness of select senescence-regulated genes in early passage (PD30) BJ fibroblasts.

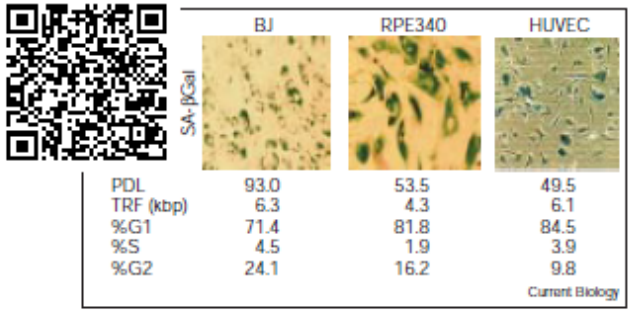
senescence response appears to overlap substantially with gene expression patterns observed in activated fibroblasts during wound healing [24–26]. MCP-1, Gro-α, IL-1β and IL-15 are strong effectors of macrophage and neutrophil recruitment and activation [27,28]. The upregulation of Toll (Tlr-4) in senescent fibroblasts confirms the overall immune response behavior at senescence. Tlr-4 is an IL-1 receptor homolog and is implicated in the activation of the gene regulatory protein NF-κB, a function proposed to be part of the innate immune response [29]. The induction of IL-15 at senescence is also consistent with an innate immune response, as IL-15 can be induced by NF-κB-dependent transcription [30] and also participates in inflammatory disease processes [28].

Deficiencies in the response of senescent cells to serum stimulation have been reported, and include an inability to induce the expression of c-*fos* mRNA [31] and markers of late G1 and S phase [32]. In response to serum, expression of inflammatory chemokines, matrix-degrading proteases and their modulators is induced in early-passage dermal fibroblasts, and expression of matrix collagens is reduced. This transient burst of activity may represent the natural response of these cells in wound repair [24]. Id-2 transcripts were hyper-induced in serum-stimulated senescent fibroblasts, which may be linked to the ability of cytokines to induce Id-2 expression [33]. The levels of mRNAs for many of these induced genes peak at a period predicted to span the G1/S boundary ([24] and this study); thus, the senescence response may mimic an activated state that states overlap substantially with those in telomere-induced senescence (W.F., D.N.S., R. Allsopp, S. Lowe, and G. Ferbeyre, unpublished observations) and thus are likely to use many of the same activation processes.

The pattern of gene expression at senescence varies substantially in different cell types. Although the expression of matrix and structural proteins, such as the collagens, keratins and auxiliary factors, is repressed in RPE cells, inflammatory regulators are not induced, in contrast to dermal fibroblasts. Physiologically, this would make sense, as an acute inflammatory response in a tissue critical for normal vision would be likely to have deleterious consequences. However, as the RPE layer has a central role in the deposition and maintenance of extracellular matrix in the retina, decrements in the ability of senescent RPE cells to maintain appropriate expression patterns, as evidenced by decreased expression of collagens, keratins, aggrecan, transglutaminase and so on, would be predicted to have adverse affects on retinal architecture. Dysfunction of the RPE cell layer is considered to be a substantial factor in the development of age-related macular degeneration [36].

Surprisingly, early-passage HUVECs overexpress many of the markers associated with senescence in dermal fibroblasts, such the pro-inflammatory molecules IL-15 and Tlr-4. In very low serum, vascular endothelial cells are often induced to undergo apoptosis, and the conditions used in this study (0.15% fetal bovine serum (FBS)) have been set to induce cessation of growth (quiescence) while

Figure 1



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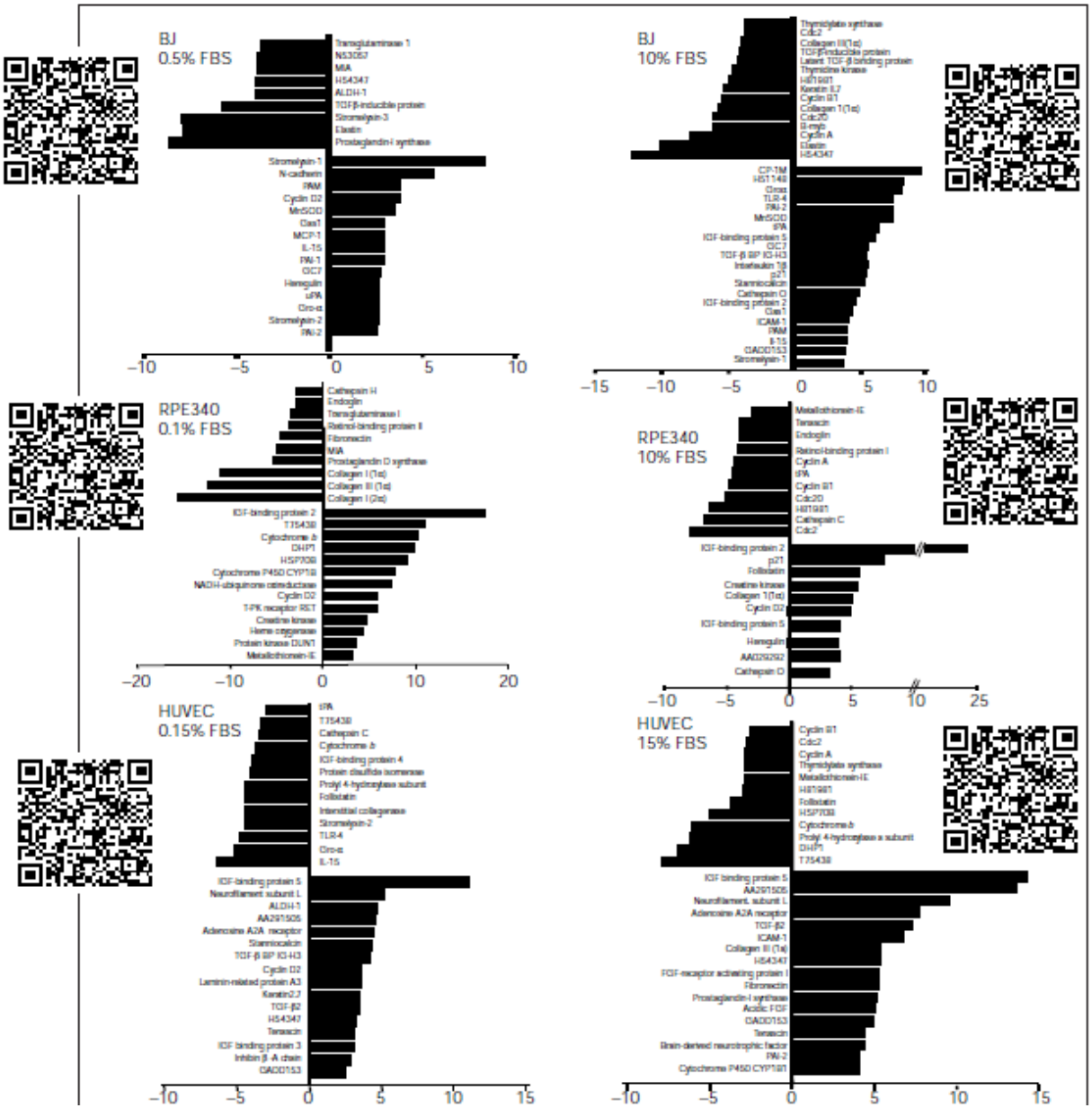

BJ
0.5% FBS
BJ
10% FBS
RPE340
0.1% FBS
RPE340
10% FBS
HUVEC
0.15% FBS
HUVEC
15% FBS

Table 1

(a) Pattern of gene expression in dermal fibroblasts at senescence.

	BJ		C4		MA	
%FBS	0.5	10	0.5	10	0.5	10
Stromelysin-1	8.2	4.1	1.0	22.0	–1.5	1.1
Stromelysin-2	2.6	2.0	1.6	5.9	1.9	1.6
Collagenase	2.0	1.7	–1.4	7.6	2.6	1.5
tPA	1.7	6.3	5.5	1.9	2.5	8.1
uPA	2.7	3.4	1.7	4.8	–1.2	1.2
MCP-1	2.9	9.0	2.7	4.3	2.5	3.4
Gro-α	2.8	6.6	1.6	5.8	2.5	1.2
IL-15	2.9	4.1	2.5	9.7	4.0	2.6
IL-1β	1.7	5.6	–1.2	3.1	1.3	1.2
Cathepsin O	2.9	4.8	4.2	3.9	2.2	4.2
Elastin	–8.4	–5.5	–4.5	–4.5	–8.3	–13.9
MnSOD	3.4	7.4	2.9	2.6	3.8	1.6
Tir-4	2.0	7.7	1.5	7.3	1.9	1.6
ICAM-1	–1.2	4.8	1.2	3.7	5.4	3.7

(b) Pattern of gene expression in retinal pigment epithelial cells at senescence.

	RPE340		RPE338		RPE341	
%FBS	0.1	10	0.1	10	0.1	10
IGF-binding protein-2	15.3	22.0	6.0	6.9	1.8	4.1
Collagen Iα2	–16.0	1.1	–10.6	–3.6	–58.0	–18.6
Collagen Iα1	–9.5	3.4	1.9	1.4	–26.5	–8.2
Collagen IIIα1	–9.8	1.4	1.9	3.3	–16.5	–4.0
Keratin I,18	1.4	–2.6	–3.8	–2.6	–8.1	–10.6
Keratin II,7	–1.0	–2.4	–18.5	–23.7	–24.8	–23.2
Transglutaminase 1	–3.5	–4.0	1.8	–1.9	–5.2	–3.0
Creatine kinase	3.6	7.3	3.9	2.3	1.6	2.8
Follistatin	–1.6	5.8	4.7	6.0	1.5	3.1
Cathepsin O	–2.1	3.0	2.5	2.7	24.3	11.7
Ceruloplasmin	1.4	1.7	1.0	3.7	14.6	7.0
Retinol-binding protein-1	–3.1	–3.2	1.2	1.2	–3.2	–4.4
Stanniocalcin	1.2	1.3	5.6	9.9	2.3	5.7
Thrombospondin 2	1.2	–3.5	–7.0	–3.3	–10.7	–16.8

RNA was prepared from three independent **(a)** dermal fibroblast or **(b)** RPE strains maintained in high or low serum. Positive numbers indicate fold-overexpression in senescent cultures; negative numbers indicate fold-overexpression in early passage cultures. See text and Supplementary material for abbreviations.

Time course of the response to serum stimulation

We compared the responses of early- and late-passage BJ fibroblasts to serum stimulation and a complete listing of responsive genes is provided as Supplementary material. In early-passage cells, late G1/early S-phase markers, such whereas in senescent cultures the response was attenuated (Figure 3a). Similarly, mRNAs for IκBα, tristetraproline (TTP), JunB and early growth response protein 1 (EGR-1) are all induced within 1 hour of stimulation in early-passage cells, whereas they are only weakly induced in senescent fibroblasts. The induction of mRNAs for connective tissue growth factor (CTGF) and mitogen-activated protein (MAP) kinase phosphatase 1 appeared unperturbed in senescent cells, indicating that substantial aspects of the early response to serum are maintained. Conversely, there was a pronounced induction of Id-2 in senescent cultures.

Several genes that are serum responsive in early-passage fibroblasts are constitutively upregulated in senescent cells. In early-passage cells, mRNAs for stromelysin-1, MCP-1, PAI-1, PAI-2 and others are induced by serum (Figure 3b). Conversely, collagen transcript levels decrease substantially following serum addition. The expression of many of the serum-inducible genes peaks near the 8 hour time point, at which time the cells would be in transit through the G1/S boundary.

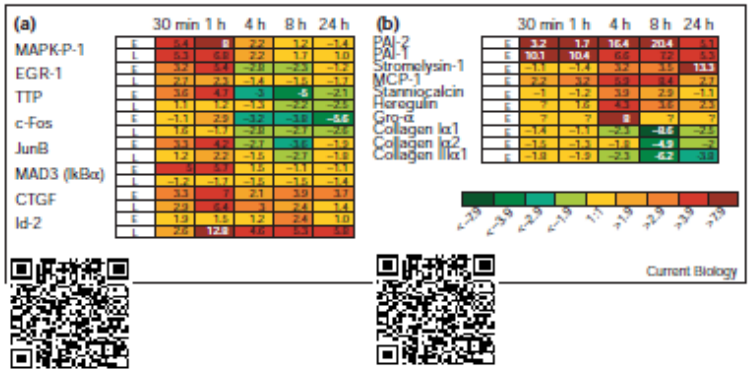
Discussion

Senescence-associated gene expression

All three cell strains reported here undergo an arrest characterized by a distinctive cell-cycle distribution, changes in cell morphology and the shortening of telomeres. Senescent cells have a cell-cycle distribution that is clearly different from that of quiescent cells, as a substantial population of cells with G2 DNA content persists [9] (Figure 2). In high-serum conditions, this arrest includes expression of the CDK inhibitor p21, and growth arrest specific protein (Gas1) in BJ fibroblasts and RPE340 strains, but not in HUVECs. The overexpression of cyclin D2 was observed in all three cell strains and may reflect a common cell-cycle braking mechanism [19,20]. Interestingly, in none of the cell strains examined did we observe an upregulation of mRNA for the CDK inhibitor p16, as reported for other cell strains [21,22], and RT–PCR analysis of mRNA from senescent BJ fibroblasts confirmed the lack of induction of mRNA for p16 (data not shown). The induction of p21 at senescence has been reported to be transient in some cell lines, whereas the induction of p16 can occur after prolonged arrest [23], and this may also account for our observed results with different cell lines. The data presented here strongly suggest that even with a common initiating signal — shortened telomeres — the ensuing arrest may be triggered by dif-

Figure 3

Time course of serum stimulation. **(a)** Early passage (E: PD30) or late passage (L: PD89) BJ cultures were held in 0.5% serum for 2 days, then stimulated with 10% FBS. RNA levels from cultures at the indicated time points (Cy5 channel) were compared with the uninduced starting culture (Cy3 channel). Positive values indicate higher expression in induced cells; negative values indicate lower expression in induced cells. Question marks indicate that there was insufficient signal for detection. A complete listing of serum-responsive genes from this analysis is provided in Supplementary material. **(b)** The serum-responsiveness of select senescence-regulated genes in early passage (PD30) BJ fibroblasts.

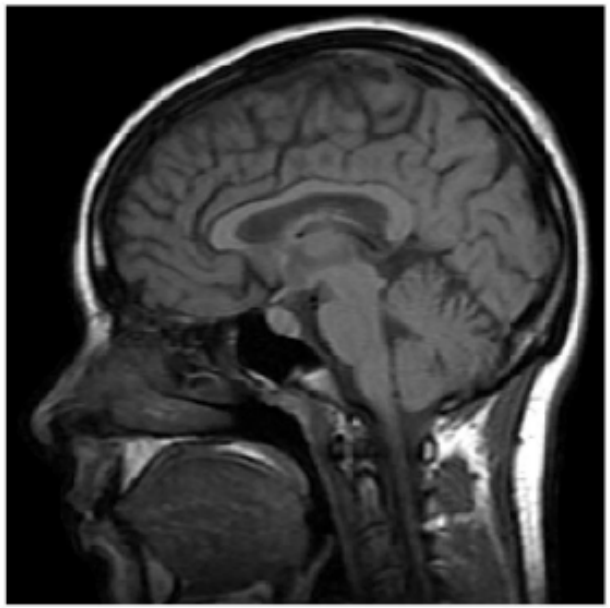


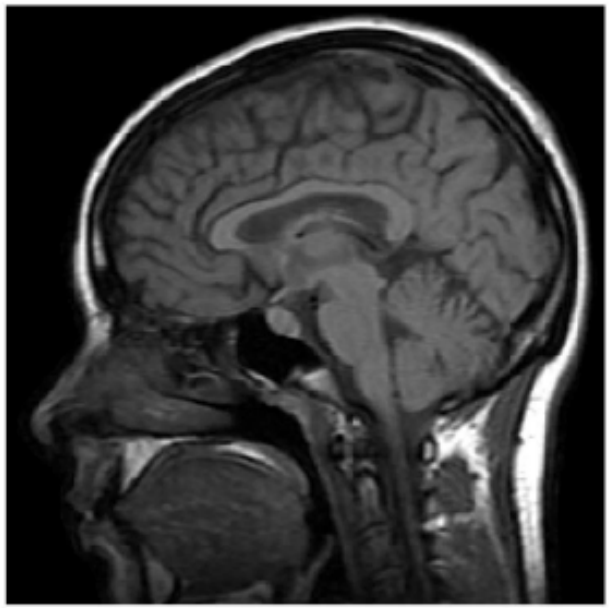
senescence response appears to overlap substantially with gene expression patterns observed in activated fibroblasts during wound healing [24–26]. MCP-1, Gro-α, IL-1β and IL-15 are strong effectors of macrophage and neutrophil recruitment and activation [27,28]. The upregulation of Toll (Tlr-4) in senescent fibroblasts confirms the overall immune response behavior at senescence. Tlr-4 is an IL-1 receptor homolog and is implicated in the activation of the gene regulatory protein NF-κB, a function proposed to be part of the innate immune response [29]. The induction of IL-15 at senescence is also consistent with an innate immune response, as IL-15 can be induced by NF-κB-dependent transcription [30] and also participates in inflammatory disease processes [28].

Deficiencies in the response of senescent cells to serum stimulation have been reported, and include an inability to induce the expression of c-*fos* mRNA [31] and markers of late G1 and S phase [32]. In response to serum, expression of inflammatory chemokines, matrix-degrading proteases and their modulators is induced in early-passage dermal fibroblasts, and expression of matrix collagens is reduced. This transient burst of activity may represent the natural response of these cells in wound repair [24]. Id-2 transcripts were hyper-induced in serum-stimulated senescent fibroblasts, which may be linked to the ability of cytokines to induce Id-2 expression [33]. The levels of mRNAs for many of these induced genes peak at a period predicted to span the G1/S boundary ([24] and this study); thus, the senescence response may mimic an activated state that states overlap substantially with those in telomere-induced senescence (W.F., D.N.S., R. Allsopp, S. Lowe, and G. Ferbeyre, unpublished observations) and thus are likely to use many of the same activation processes.

The pattern of gene expression at senescence varies substantially in different cell types. Although the expression of matrix and structural proteins, such as the collagens, keratins and auxiliary factors, is repressed in RPE cells, inflammatory regulators are not induced, in contrast to dermal fibroblasts. Physiologically, this would make sense, as an acute inflammatory response in a tissue critical for normal vision would be likely to have deleterious consequences. However, as the RPE layer has a central role in the deposition and maintenance of extracellular matrix in the retina, decrements in the ability of senescent RPE cells to maintain appropriate expression patterns, as evidenced by decreased expression of collagens, keratins, aggrecan, transglutaminase and so on, would be predicted to have adverse affects on retinal architecture. Dysfunction of the RPE cell layer is considered to be a substantial factor in the development of age-related macular degeneration [36].

Surprisingly, early-passage HUVECs overexpress many of the markers associated with senescence in dermal fibroblasts, such the pro-inflammatory molecules IL-15 and Tlr-4. In very low serum, vascular endothelial cells are often induced to undergo apoptosis, and the conditions used in this study (0.15% fetal bovine serum (FBS)) have been set to induce cessation of growth (quiescence) while





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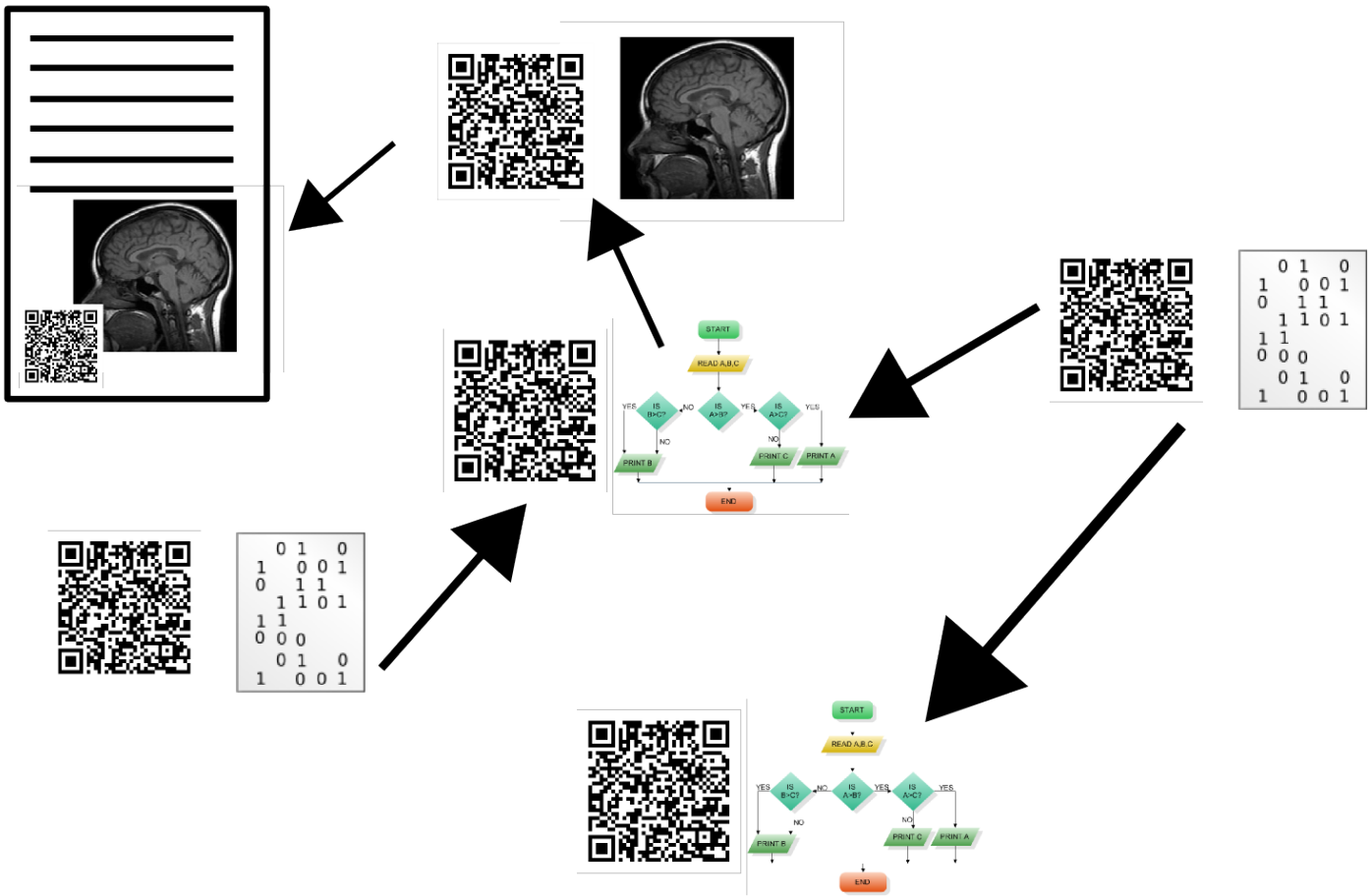
VRI's Everywhere!



Reproducibility standards in experimental sciences

A scientific experiment has:

- Initial conditions
- Specified steps
- Observed results

Lab journal: Reproducility standard for a scientific experiment

Detailed account of initial conditions and steps leading to published results

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RR efforts since 1990: Re-execute computation

- Not { realistic, easy to adopt, real benefits }
- Ignores the scientific tradition of chronicling experiments
- Ignores the hard truth: for acadmics, publication is the endgame

- The all-importnat publication is *informal* description of work we do in *private*
- Reproducibility involves cleaning up post-facto.
  It's a *pretentious waste of time*. (–DLD, 2010)

Alternative approach: Verifiable Computational Results

Automatically record, validate run-time steps leading to publishable results

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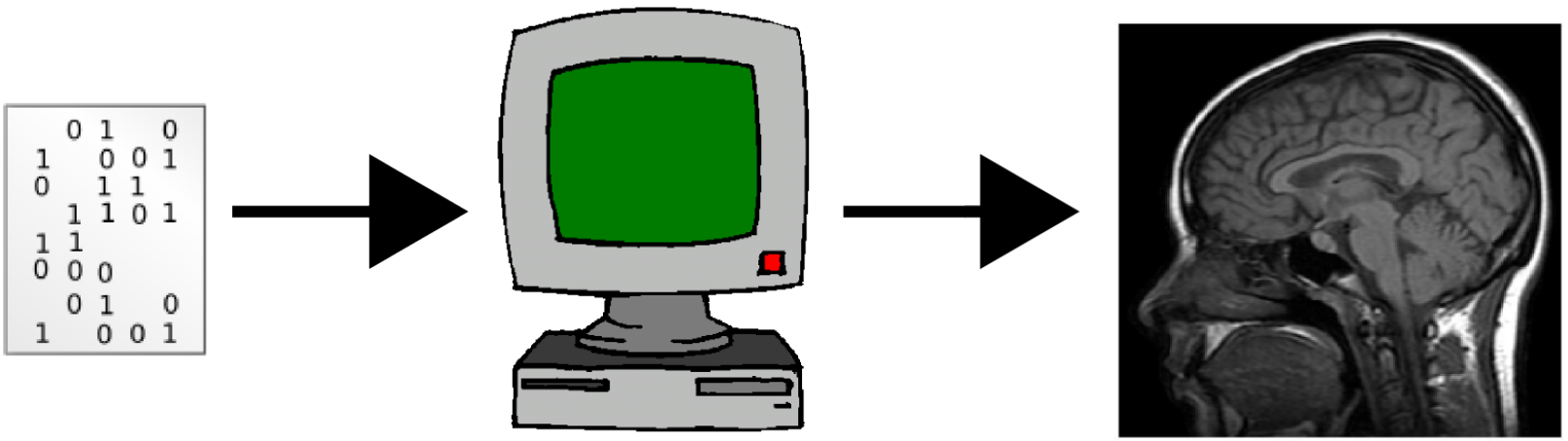

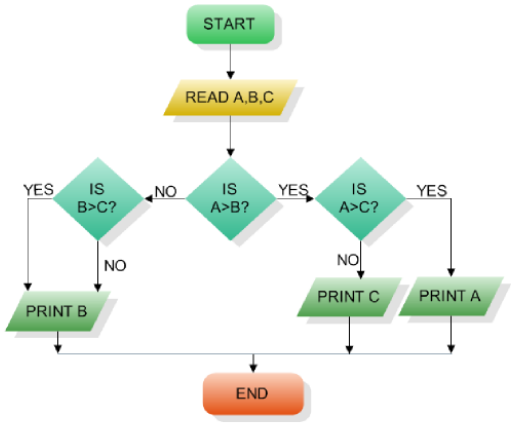
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PRINT C
PRINT A
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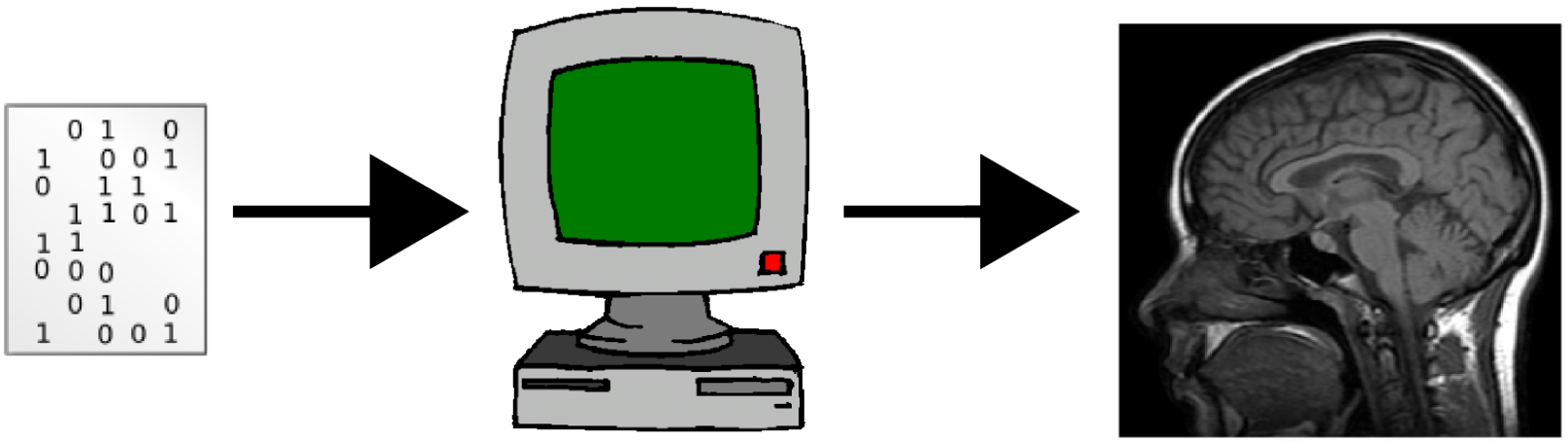

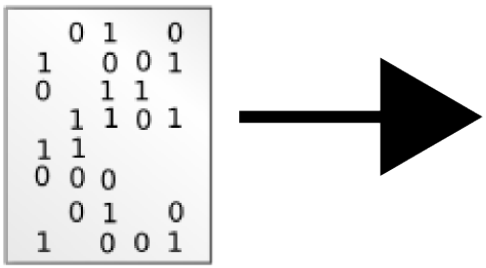

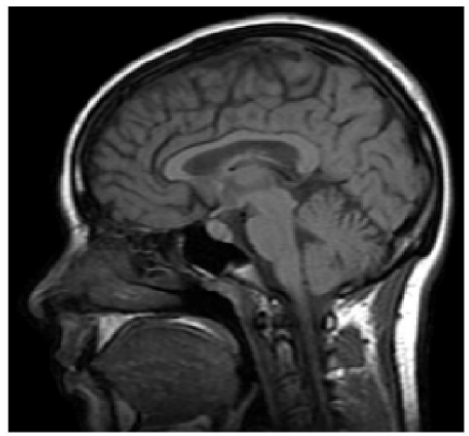

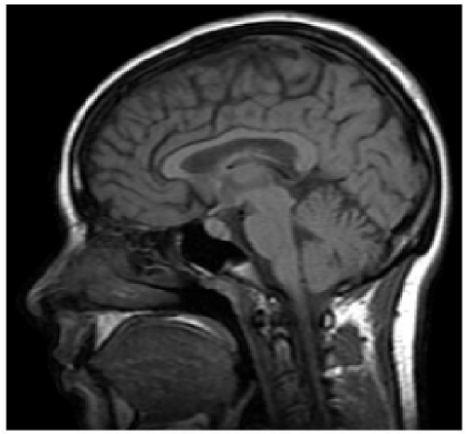

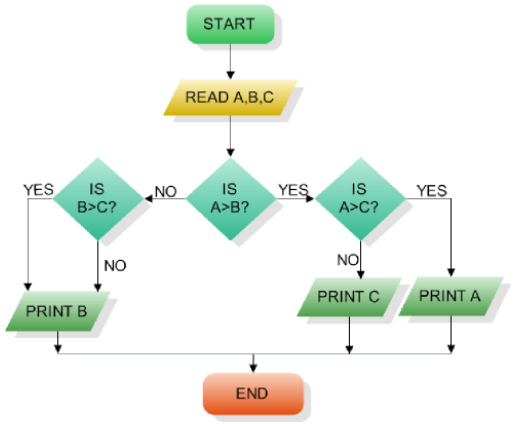
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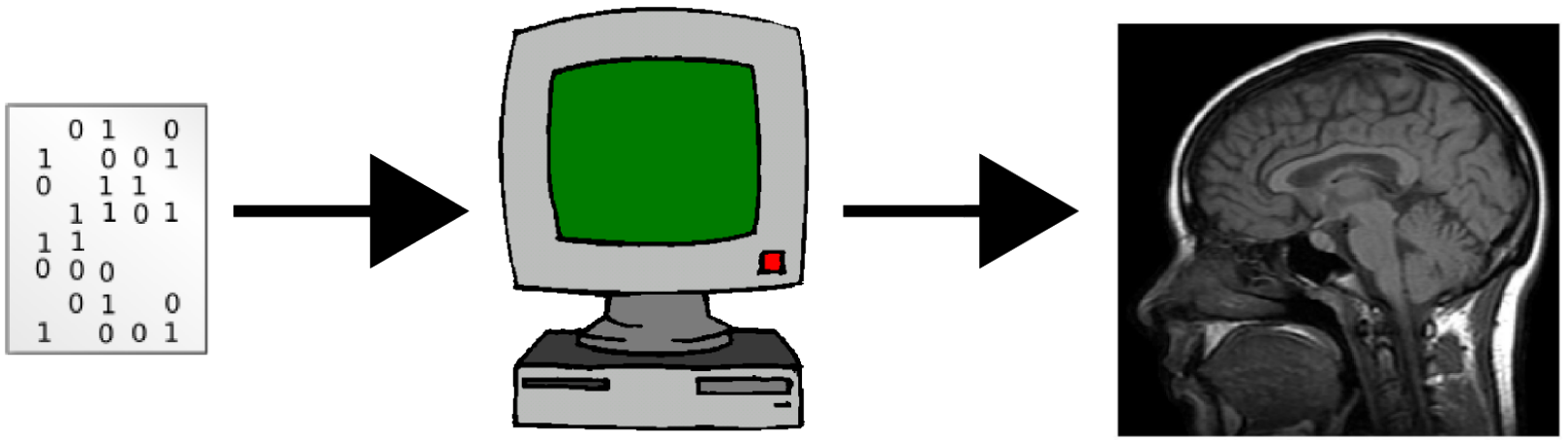

VCR record of a computational experiment

Includes –

- Code
- Original data (standard format)
- Parameters used in run-time
- Publishable & intermediate results (standard format)
- Dependencies

VCR records are permanent and citable. Peers reference it in articles, computer programs, databases.

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Outline

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1. The VCR Foundation and API
2. Chronicing computations
3. Basic VCR apps

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VCR Foundation system

(e.g vcr-stat.stanford.edu)

VCR repository server

Verifiable Result Identifier (VRI)

Addressing standard: URL, secure digital signature and QR barcode

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VCR Application Programmer Interface (API)

RESTful interface for depositing, searching and retrieving content on repositories

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GET https://vcr.cell.com/ffaaffb148d7/graphics?format=eps
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Program code with VCR plugin

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repository vcr.nature.com
verifiable figure1 = plot(x)
```

```
> vcr.nature.com approved:
> access figure1 at https://vcr.nature.com/ffaaffb148d7
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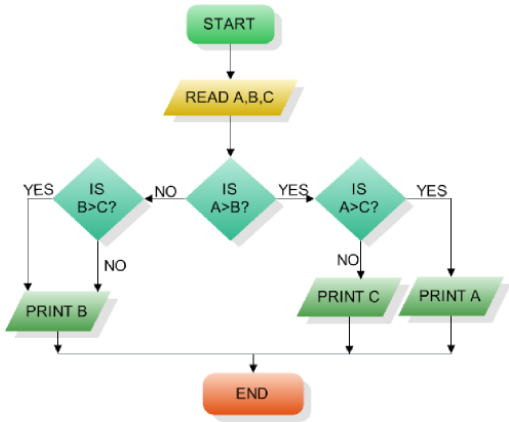
matlab plugin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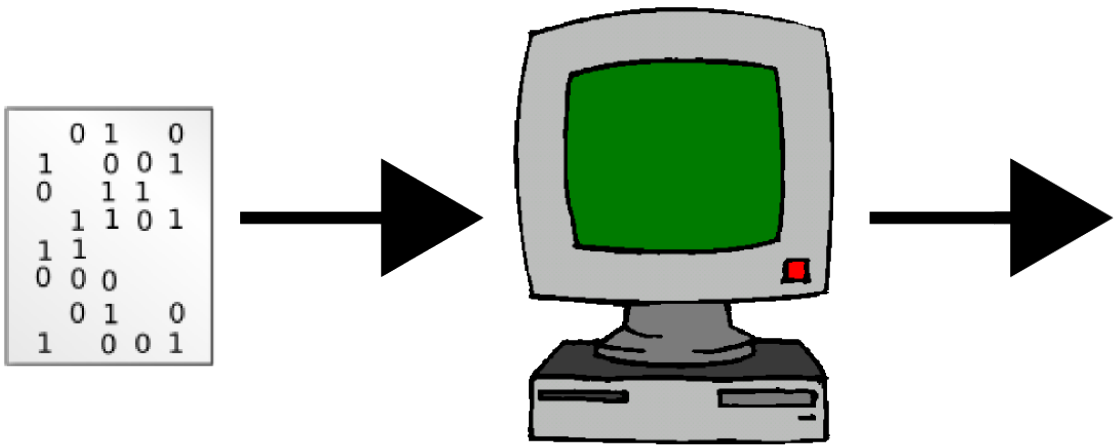

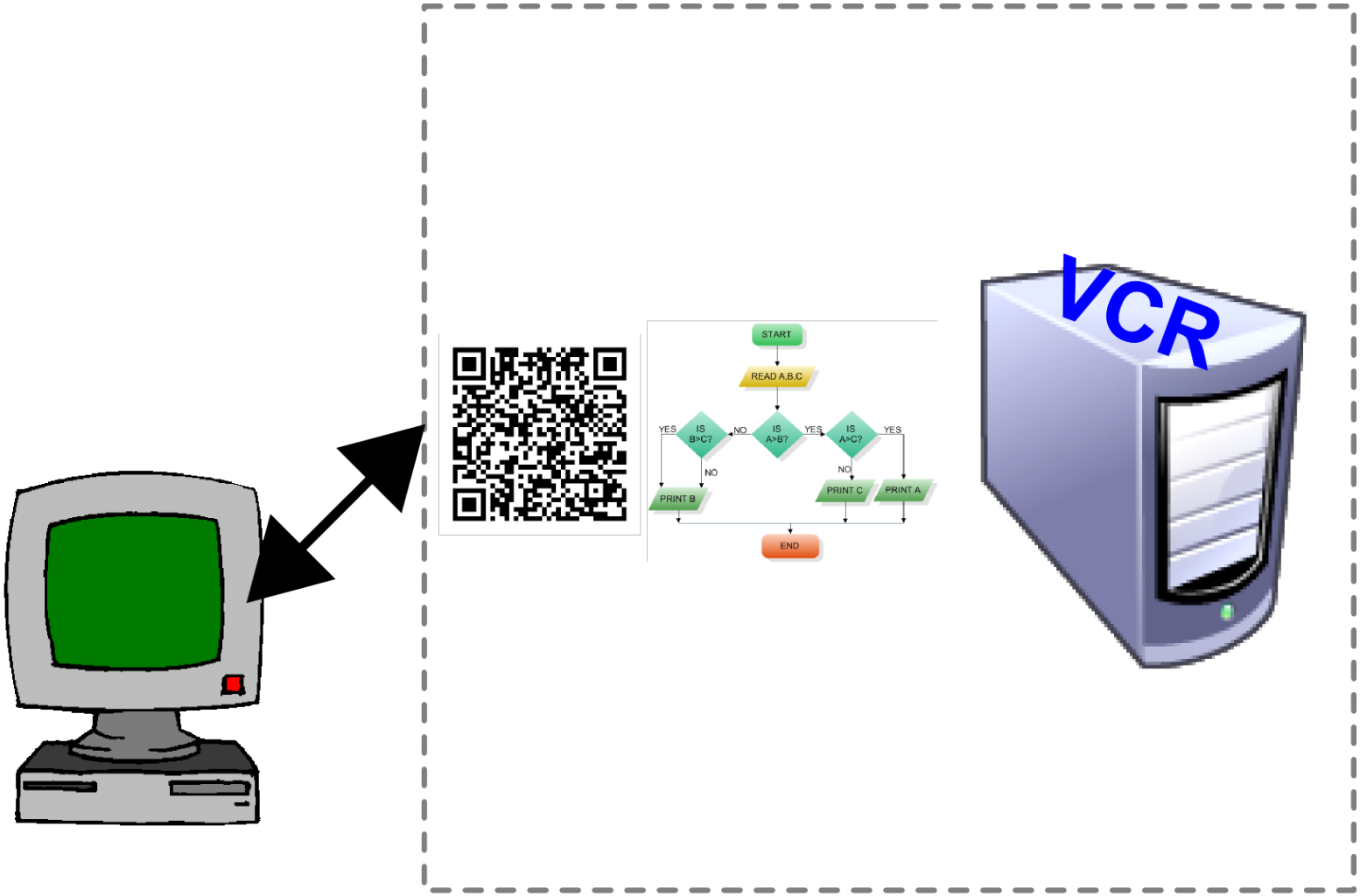
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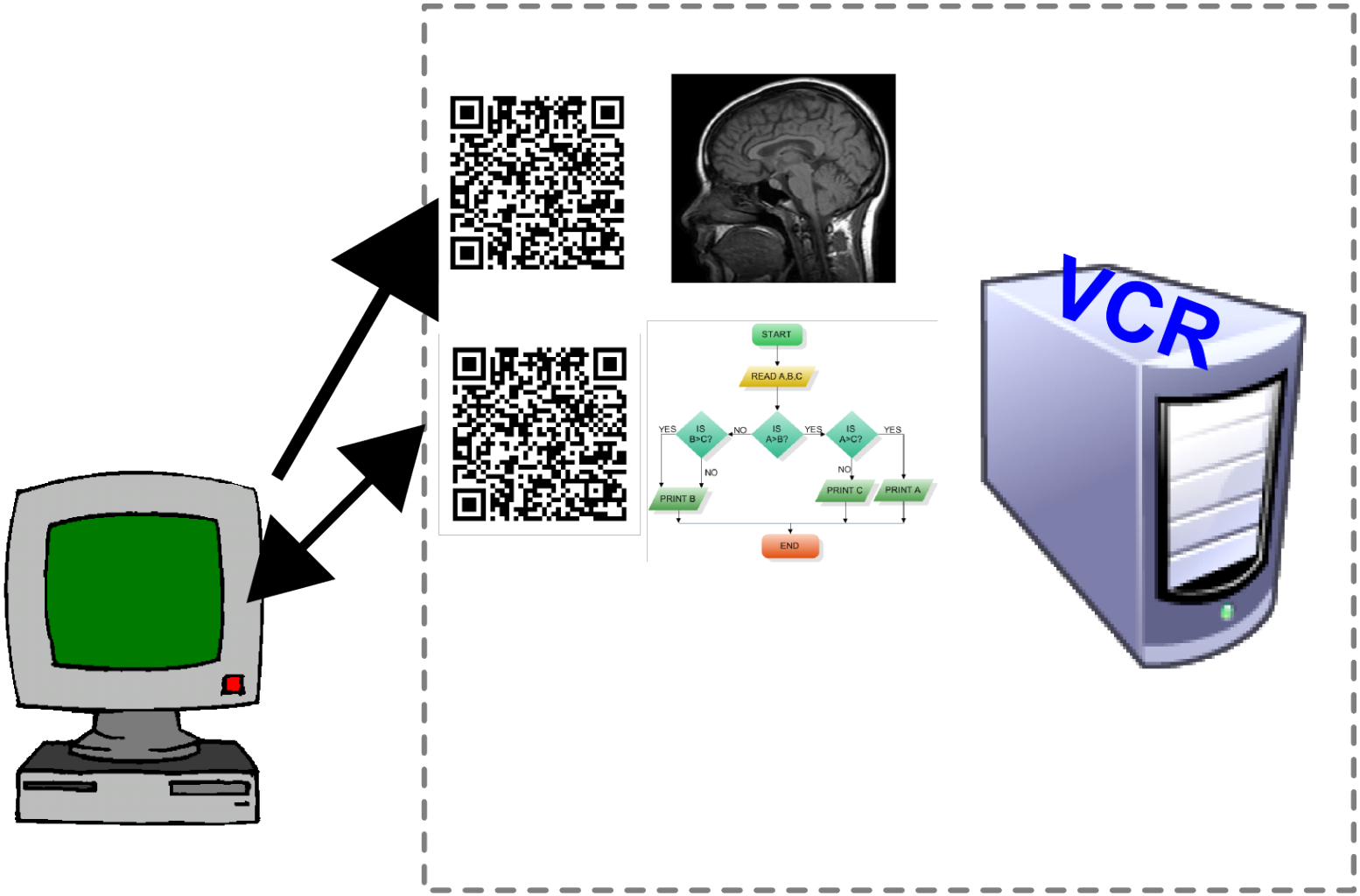
PRINT A
END
VCR


START
READ A,B,C
IS B>C?
IS A>B?
IS A>C?
YES
NO
YES
YES
NO
NO
PRINT B
PRINT C
PRINT A
END
VCR

Converting computation result into content in real-time

- Data/code published at run-time, no intervention possible or required
- Researcher controls granularity, can choose "private VRI"
- Repository automatically validates, signs computation result content

Converting computation result into content in real-time

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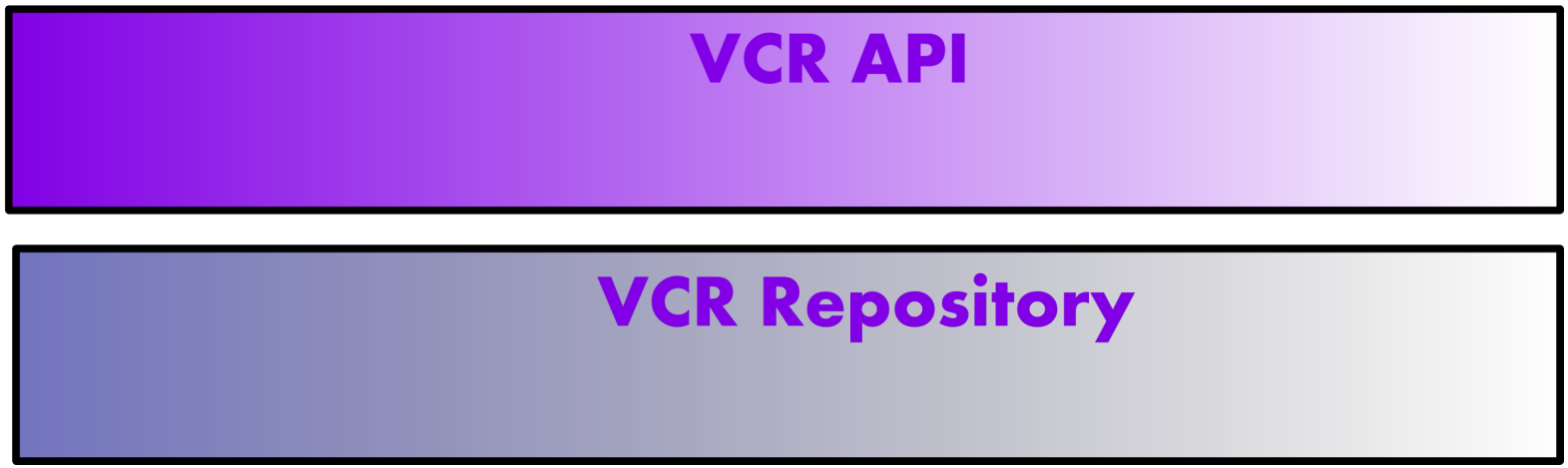
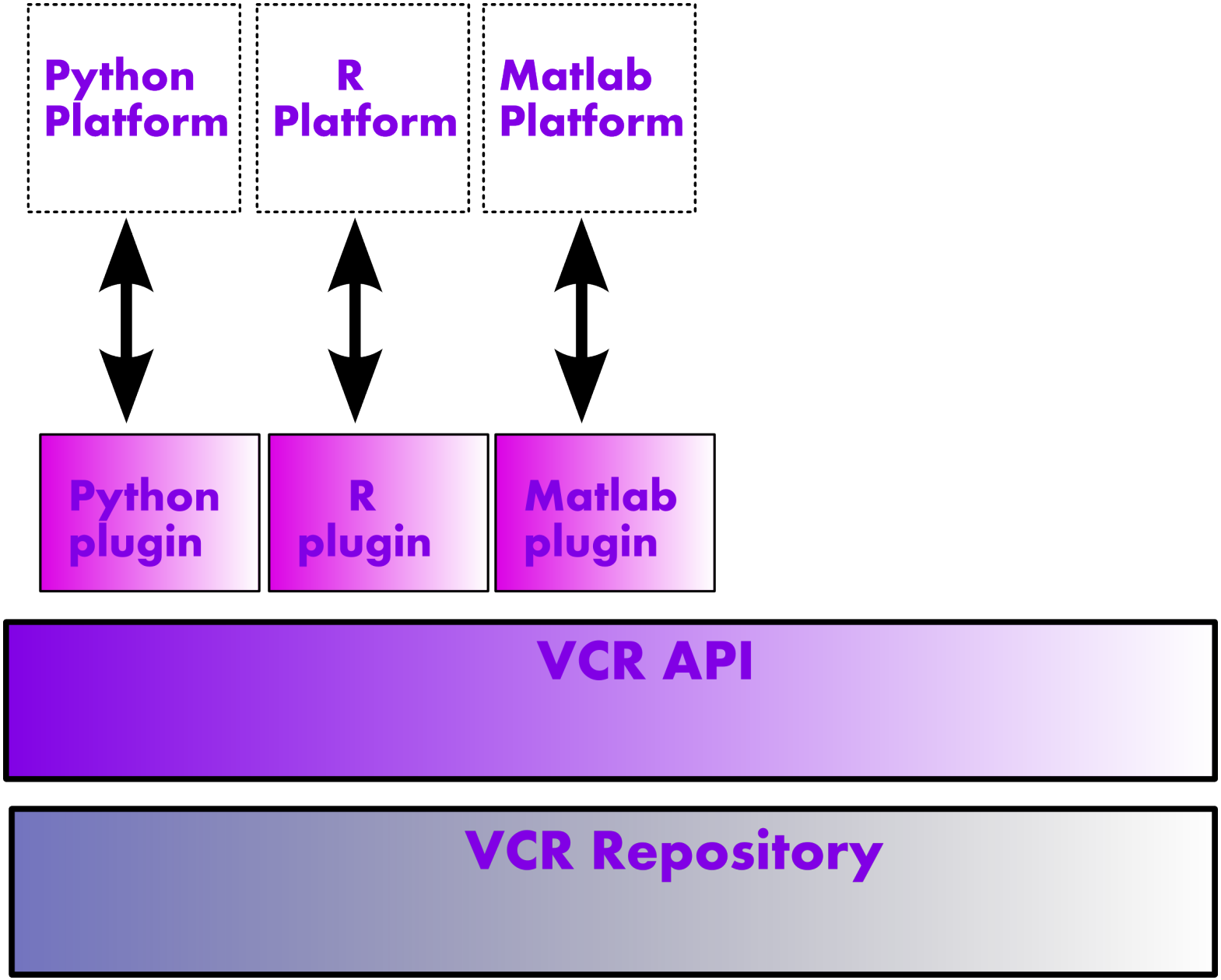
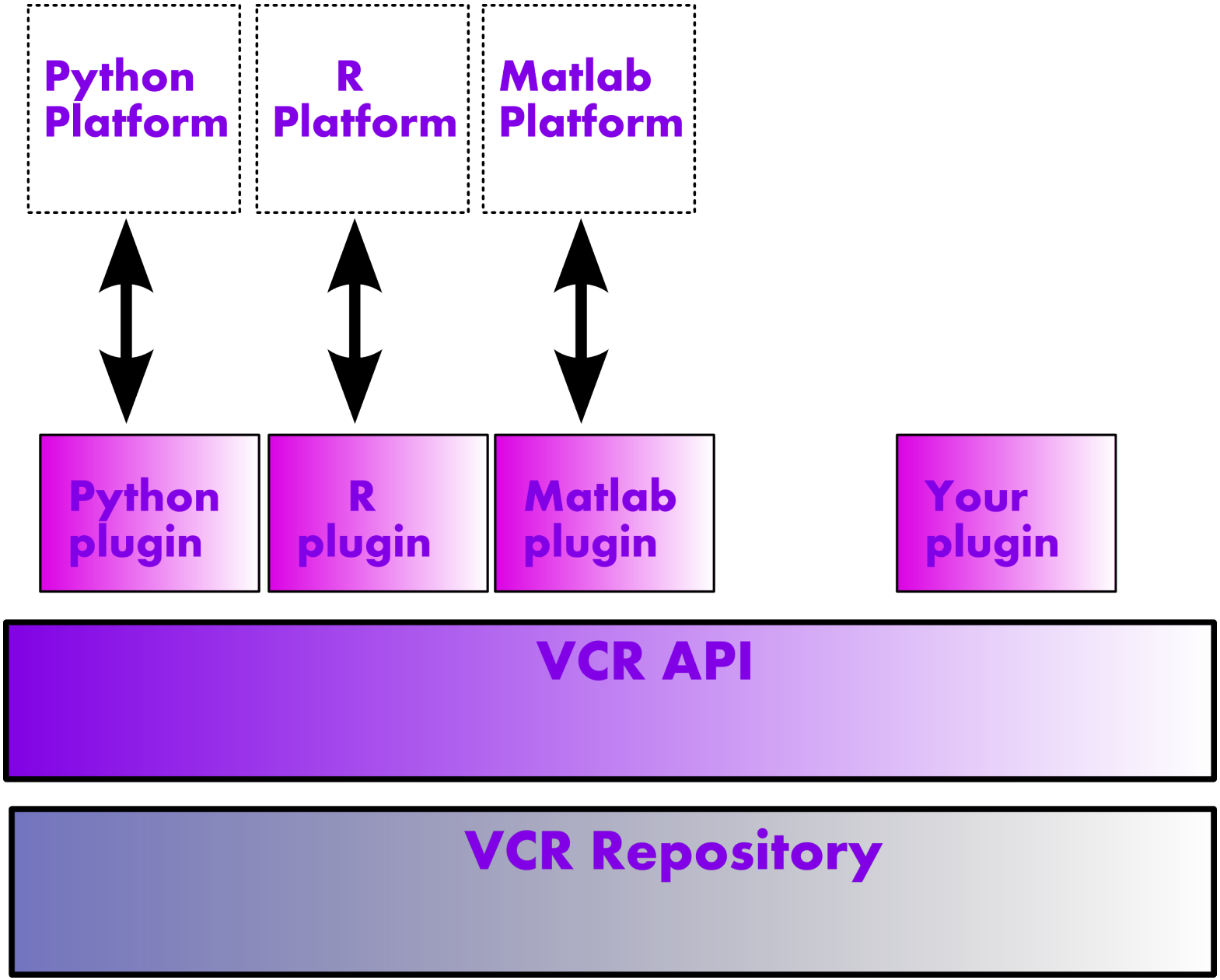
VCR foundation software



VCR foundation software



VCR foundation software



Outline

1. The VCR Foundation and API
2. Chronicing computations
3. Basic VCR apps

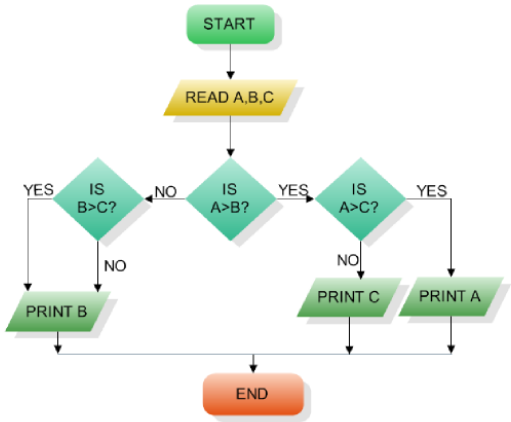
Outline

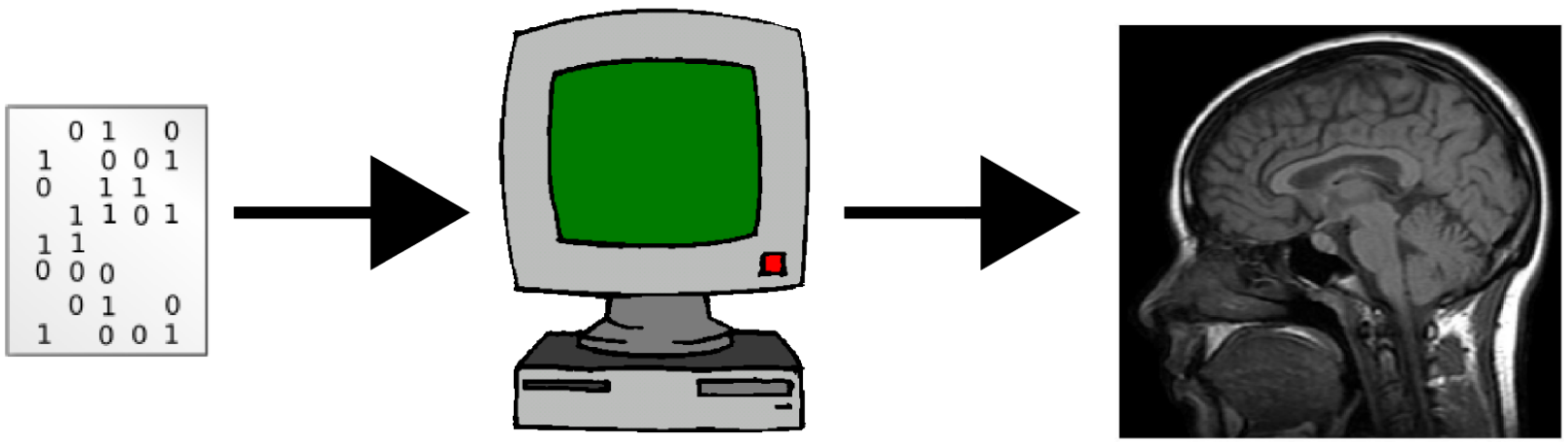
Basic VCR apps

- Direct data import
- Word-processor plugin
- Content browser
- Search

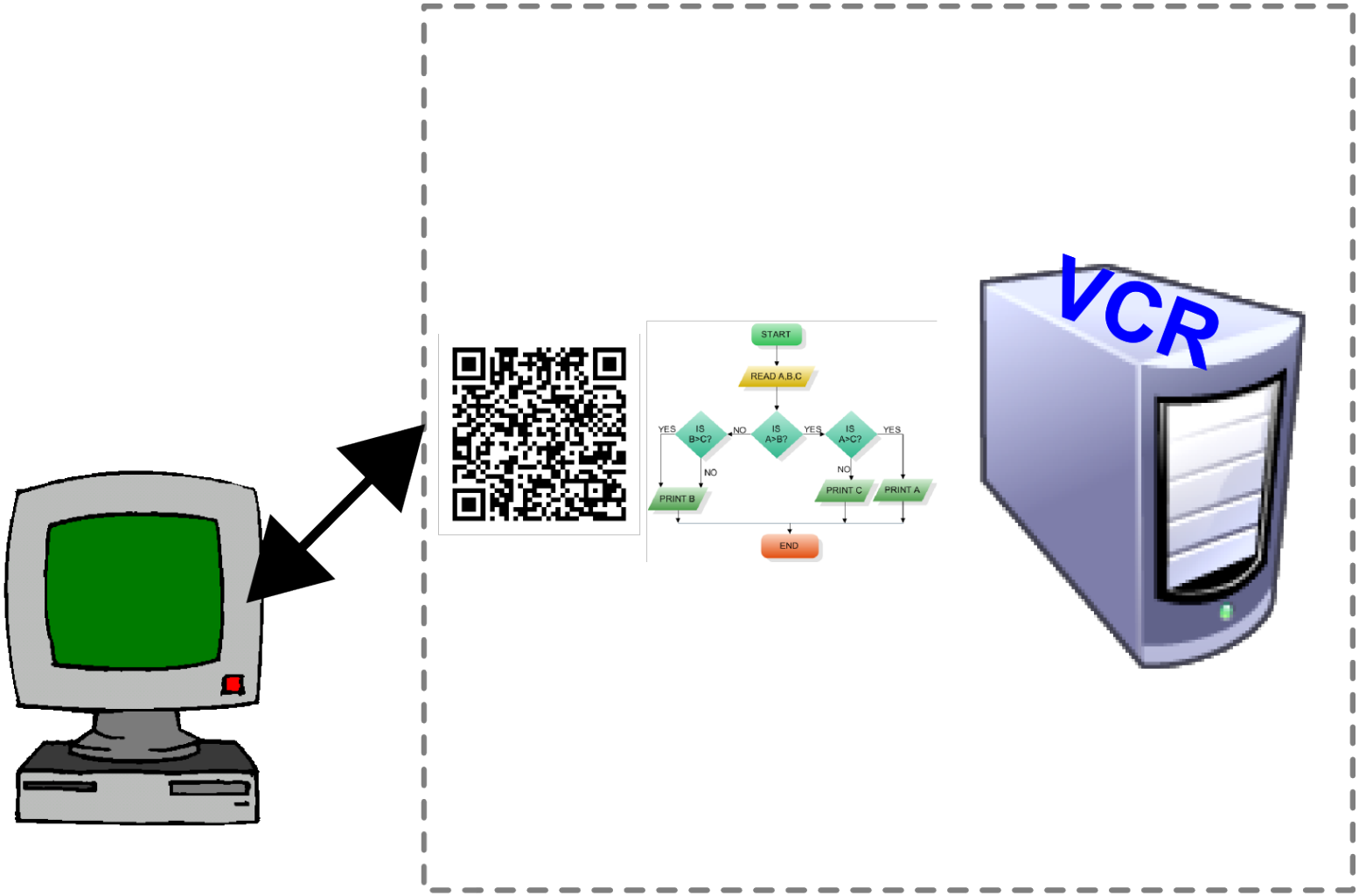
Direct data import App

Direct data import App

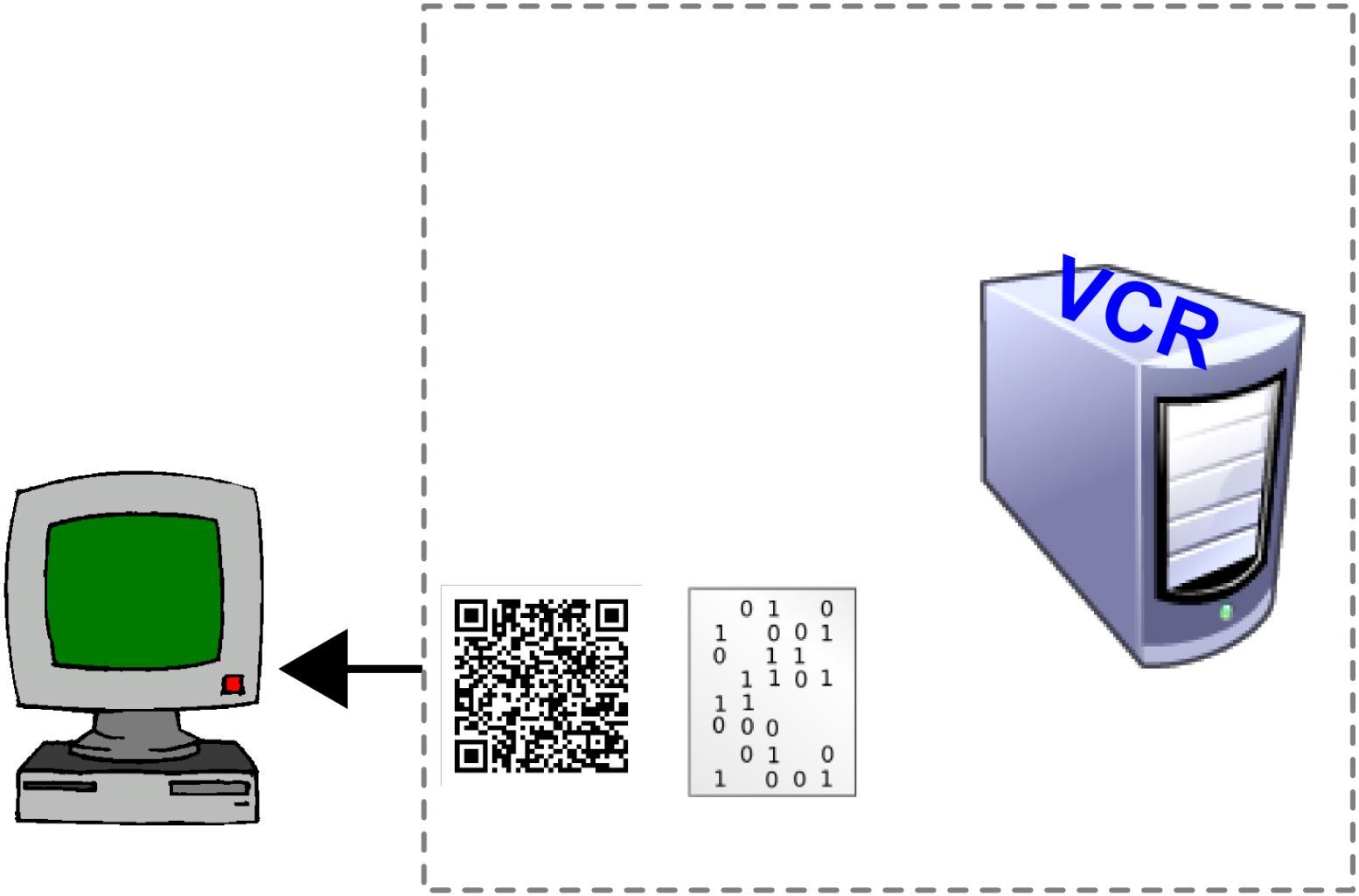




Direct data import App



Direct data import App



Direct data import App

Program Code

```
x = load('genome.csv')
run_experiment(x)
```

Program Code with VCR plugin

```
run_experiment([vcr.cell.com/ffaaffb148d7])
```

A natural evolution of scholarly citation

Direct data import App

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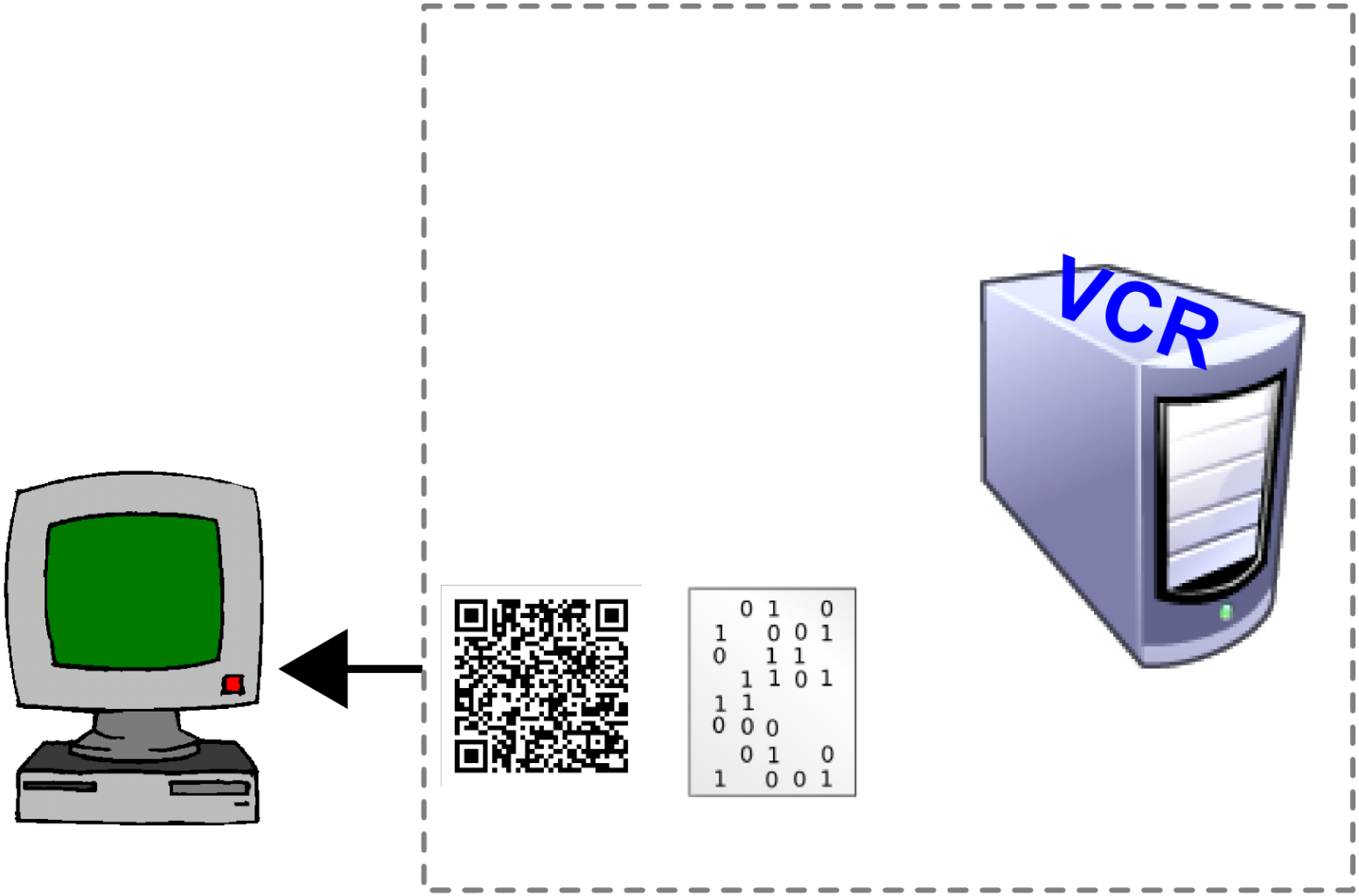

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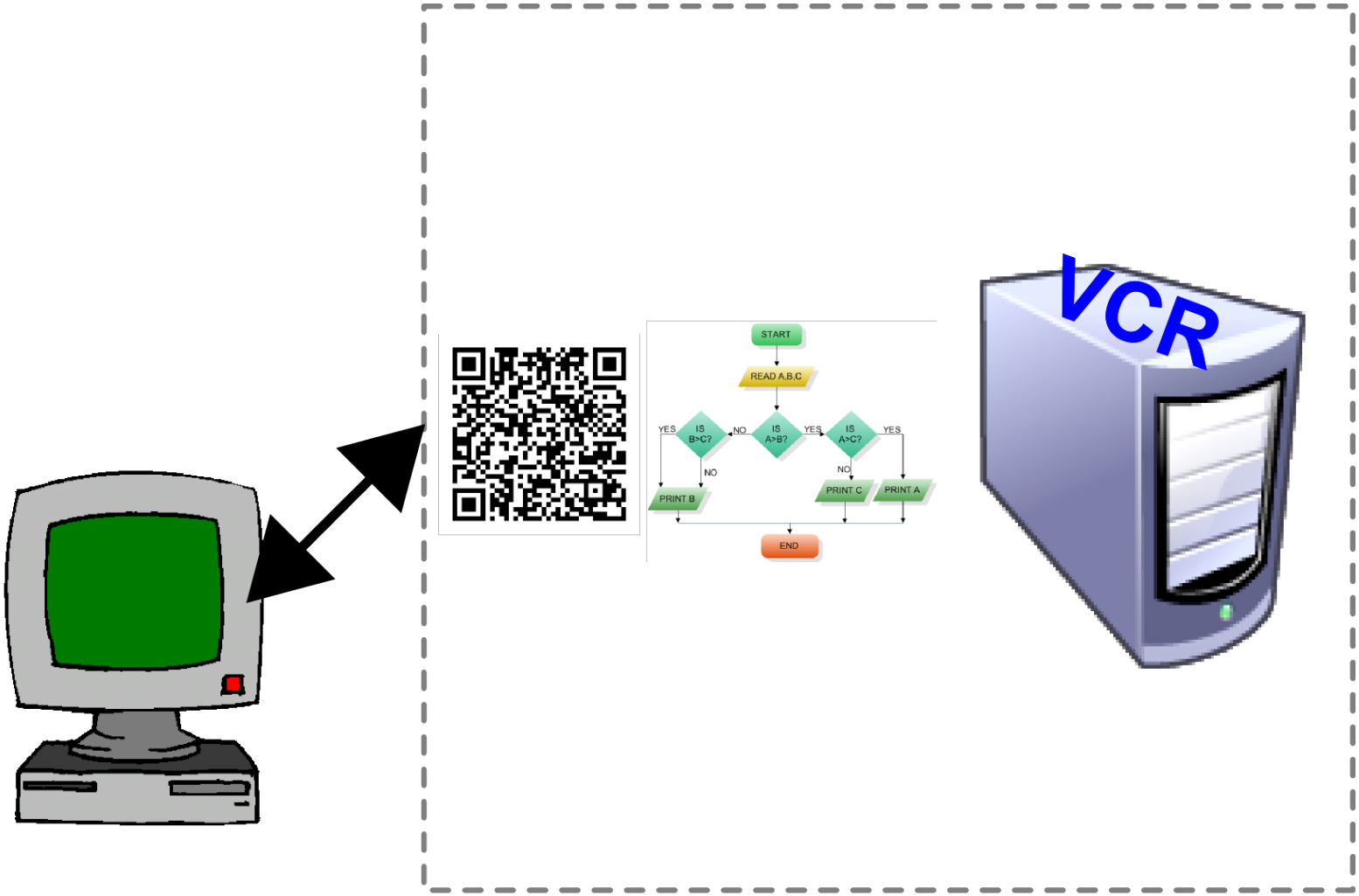

Word-processor plugin App

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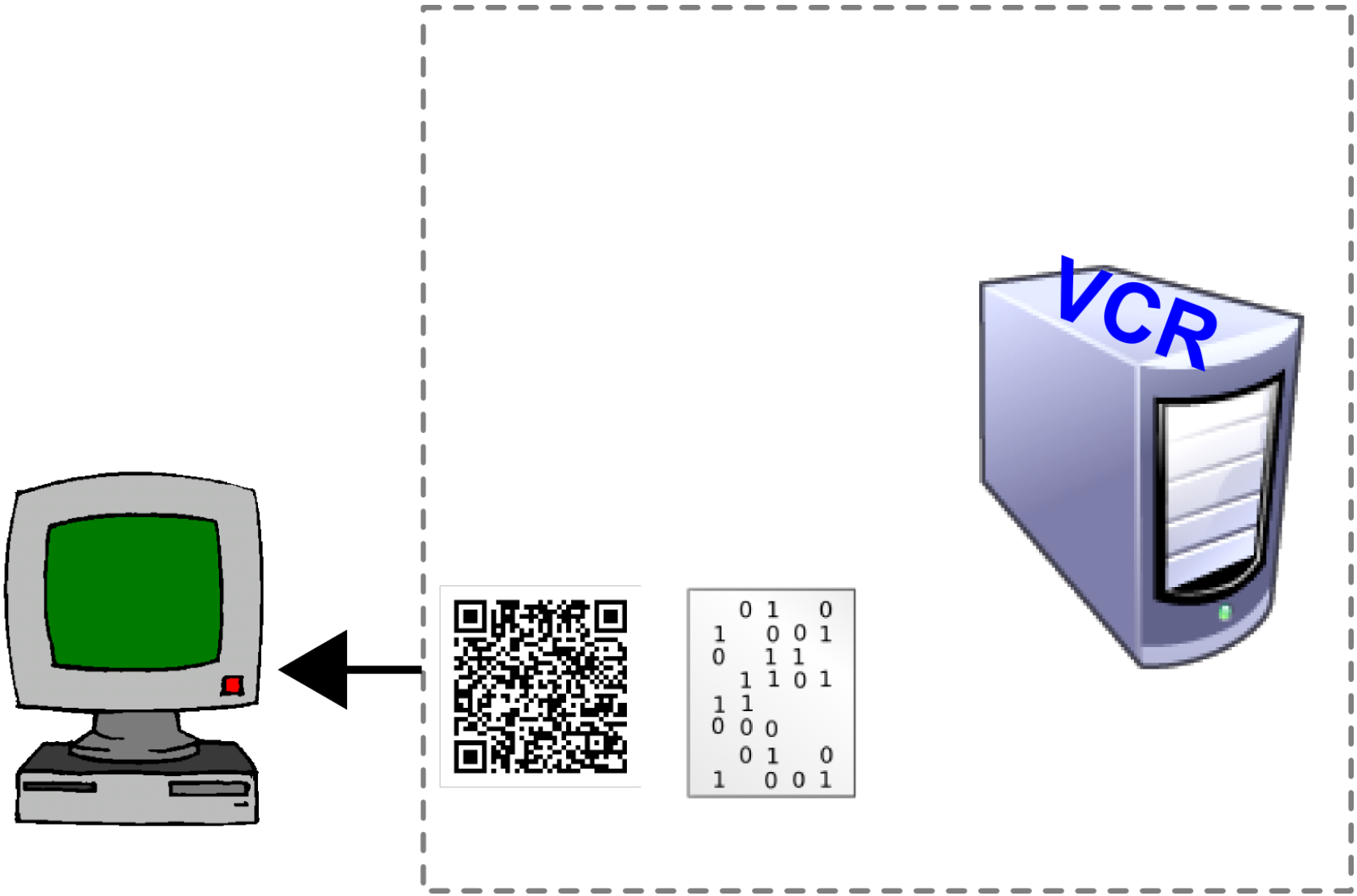

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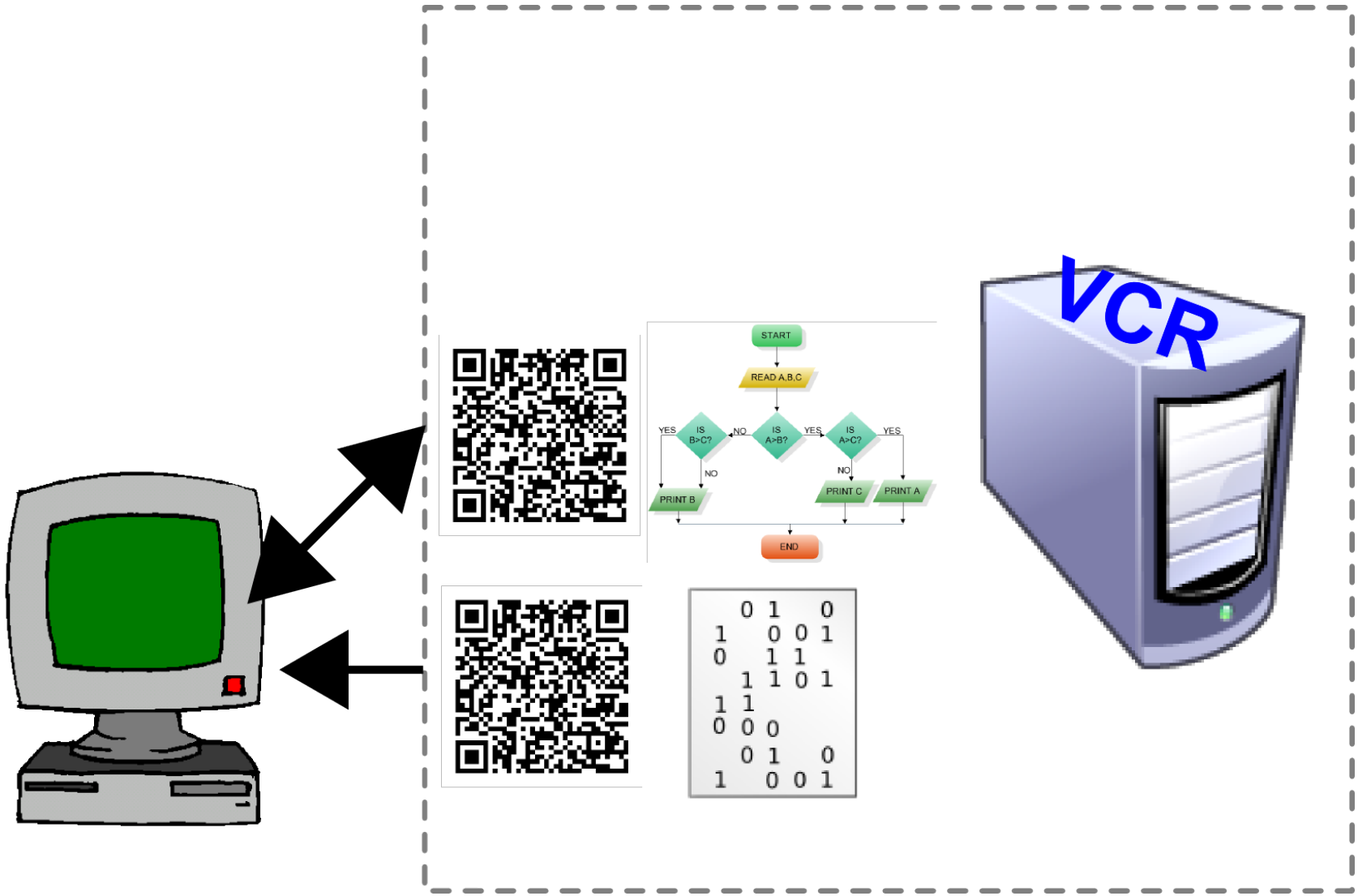

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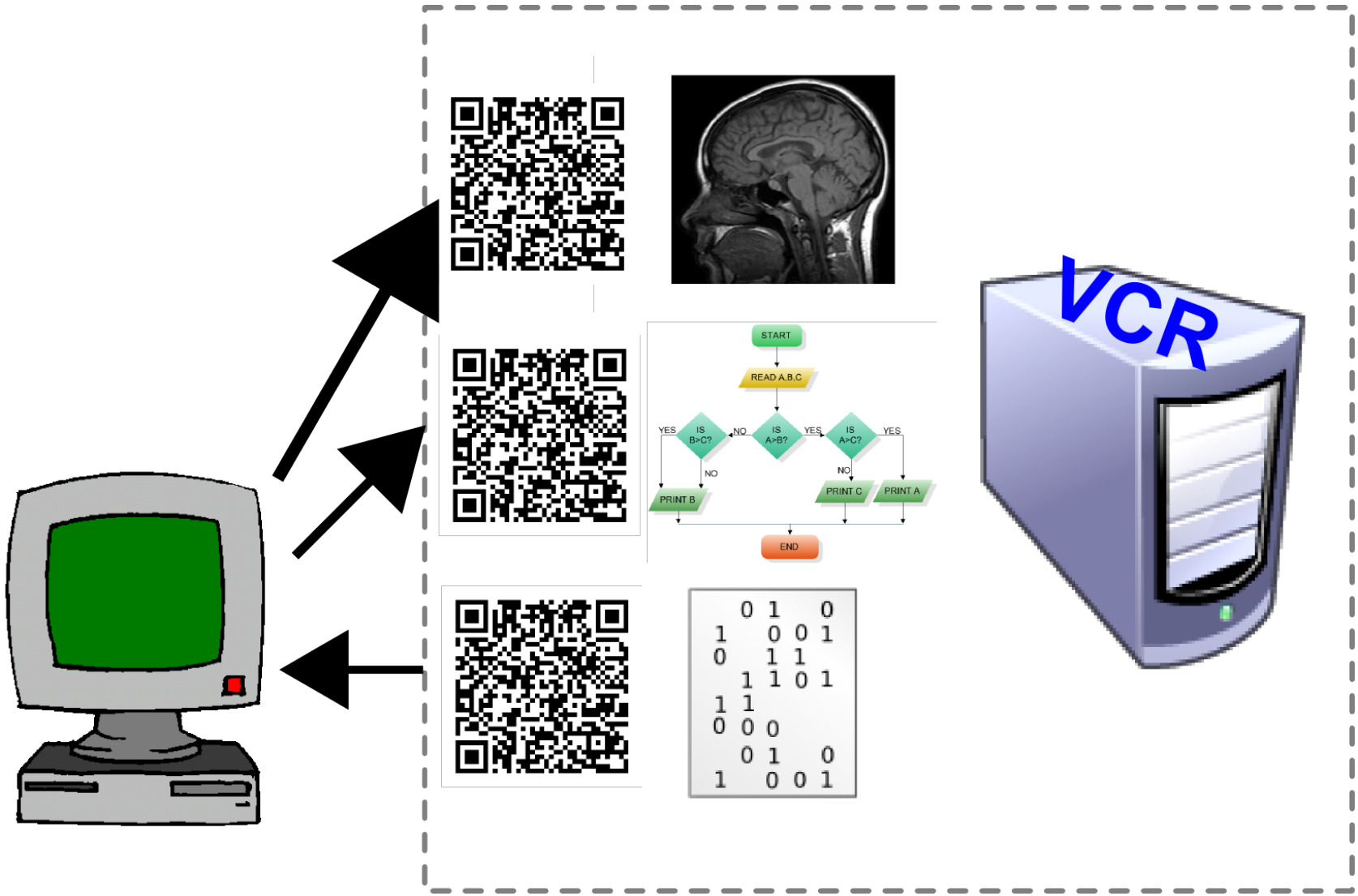

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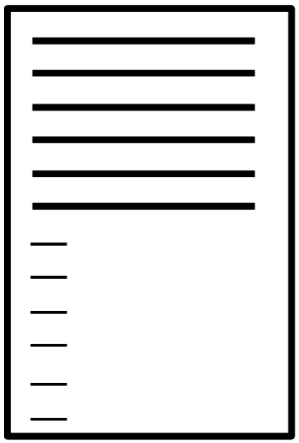

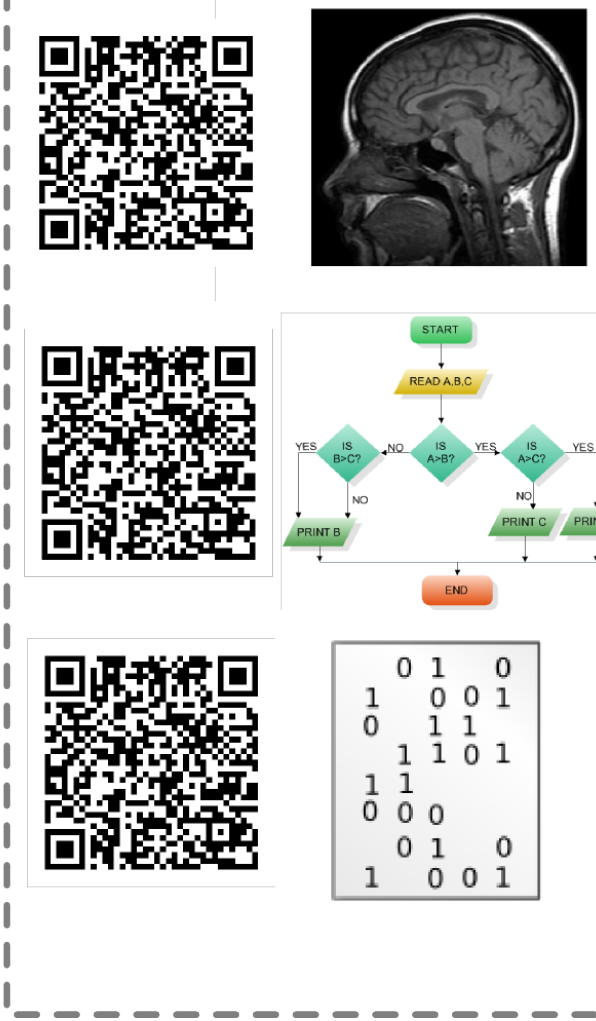

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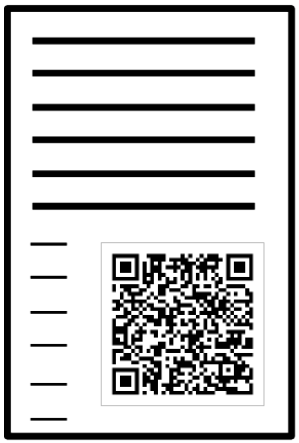
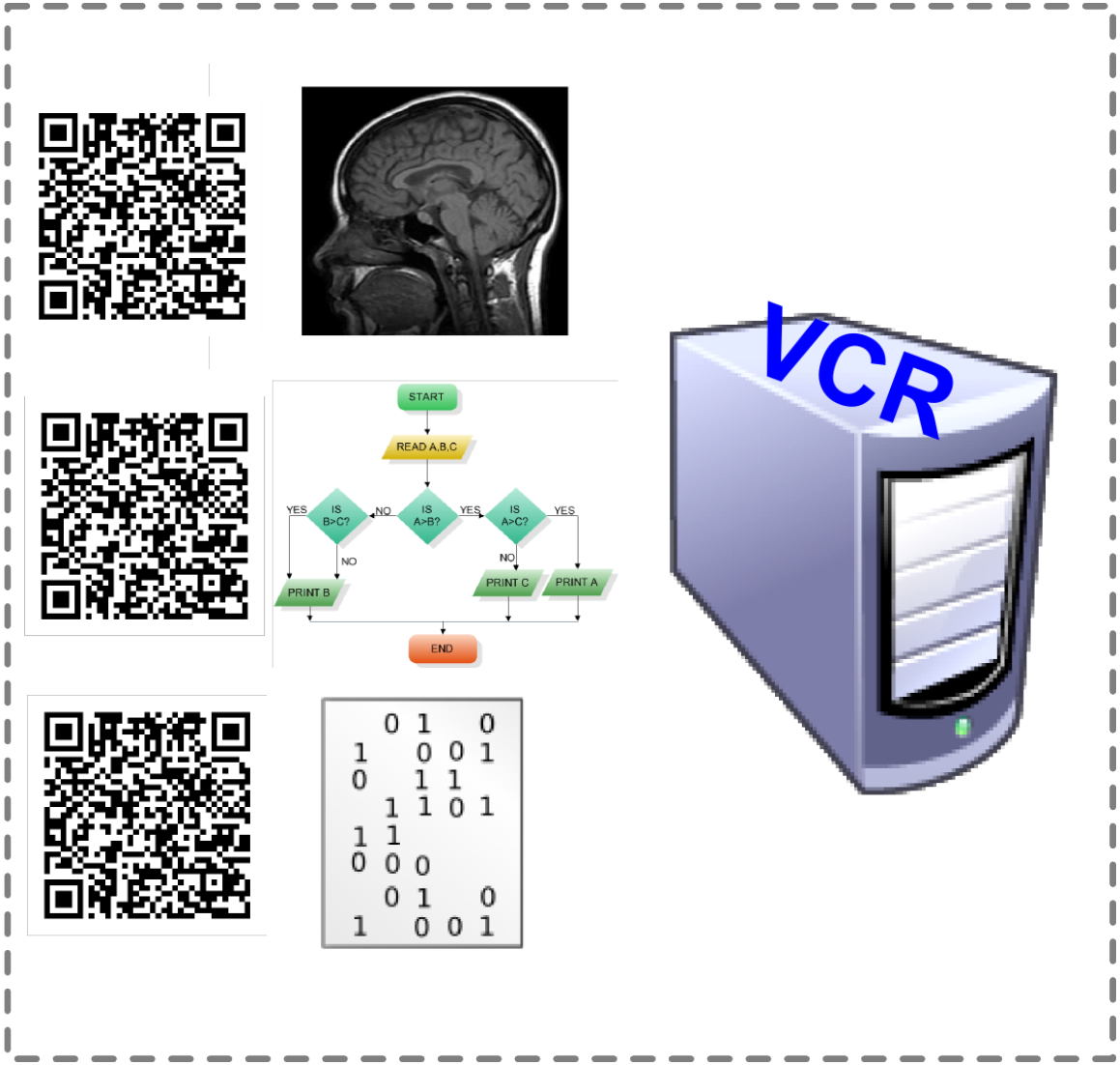
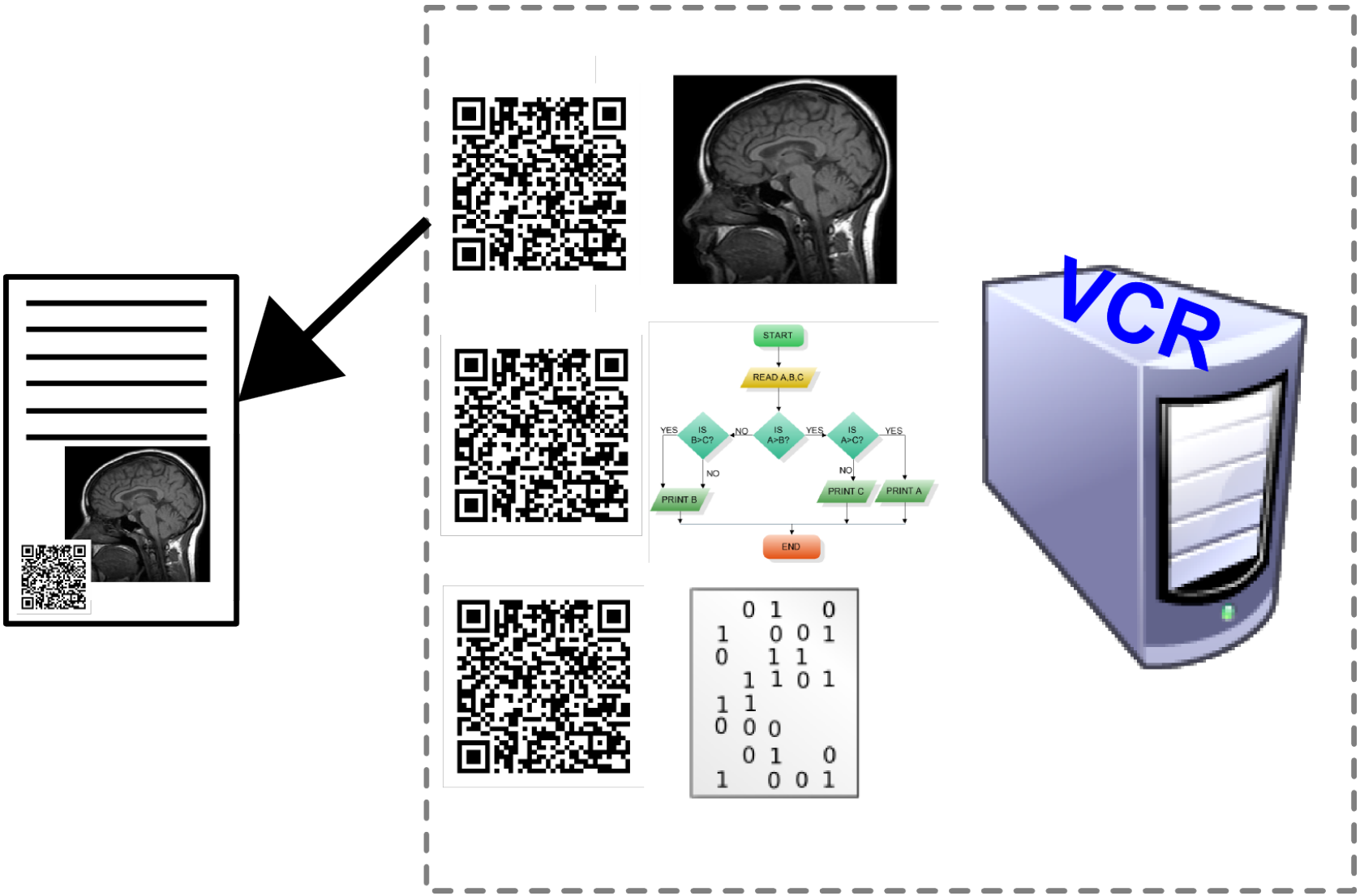




Word-processor plugin App



Word-processor plugin App

LaTeX source

```
\includegraphics{figure1.eps}
```

LaTeX source with VCR package

```
\includeresult{vcr.thelancet.com/ffaaffb148d7}
```

Permanently bind printed graphics to underlying result content

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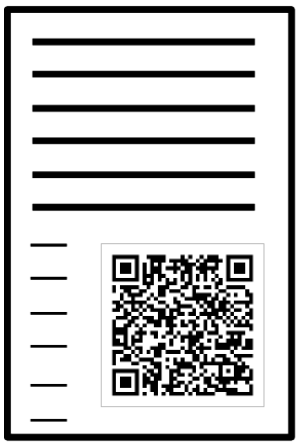

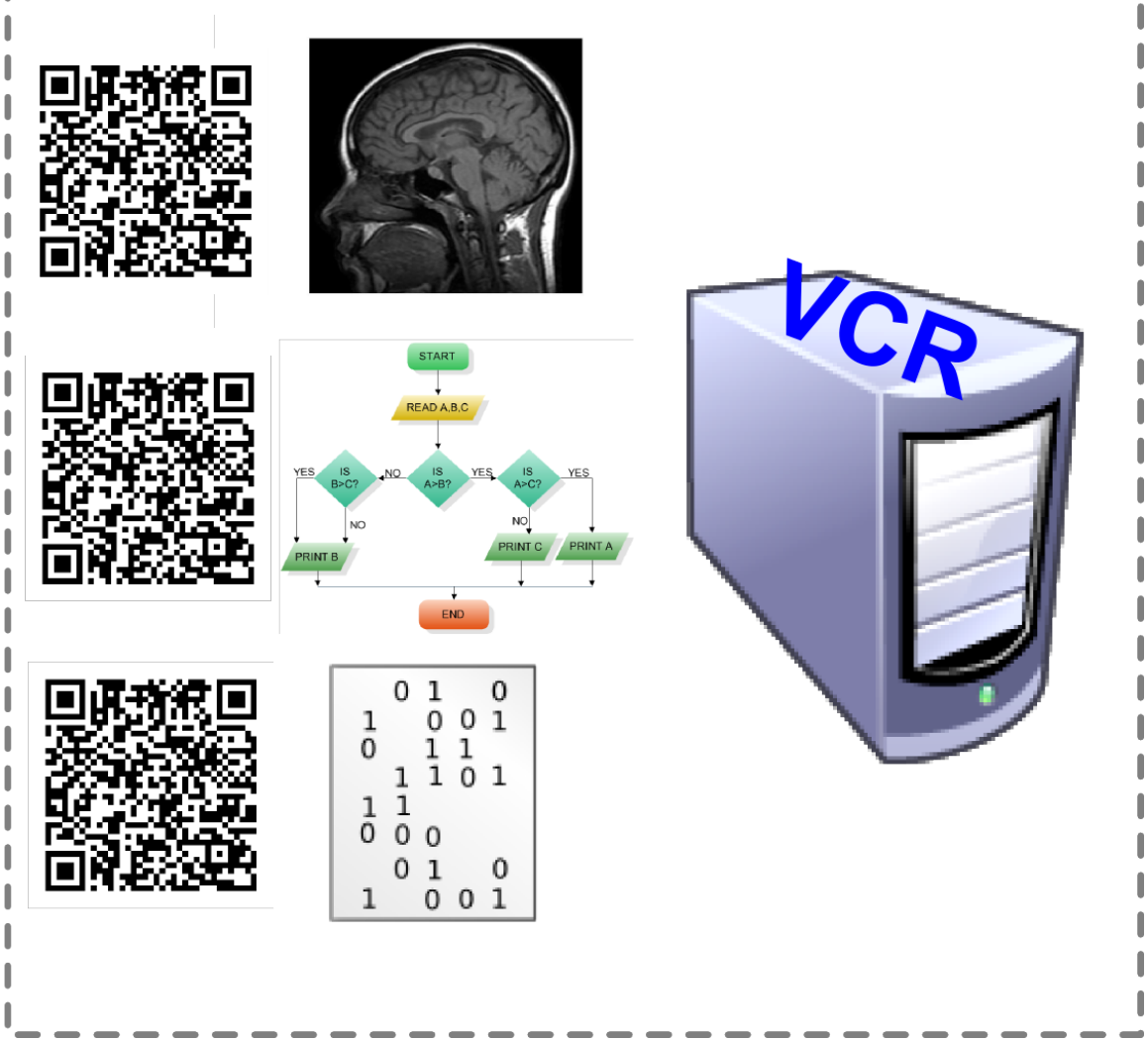

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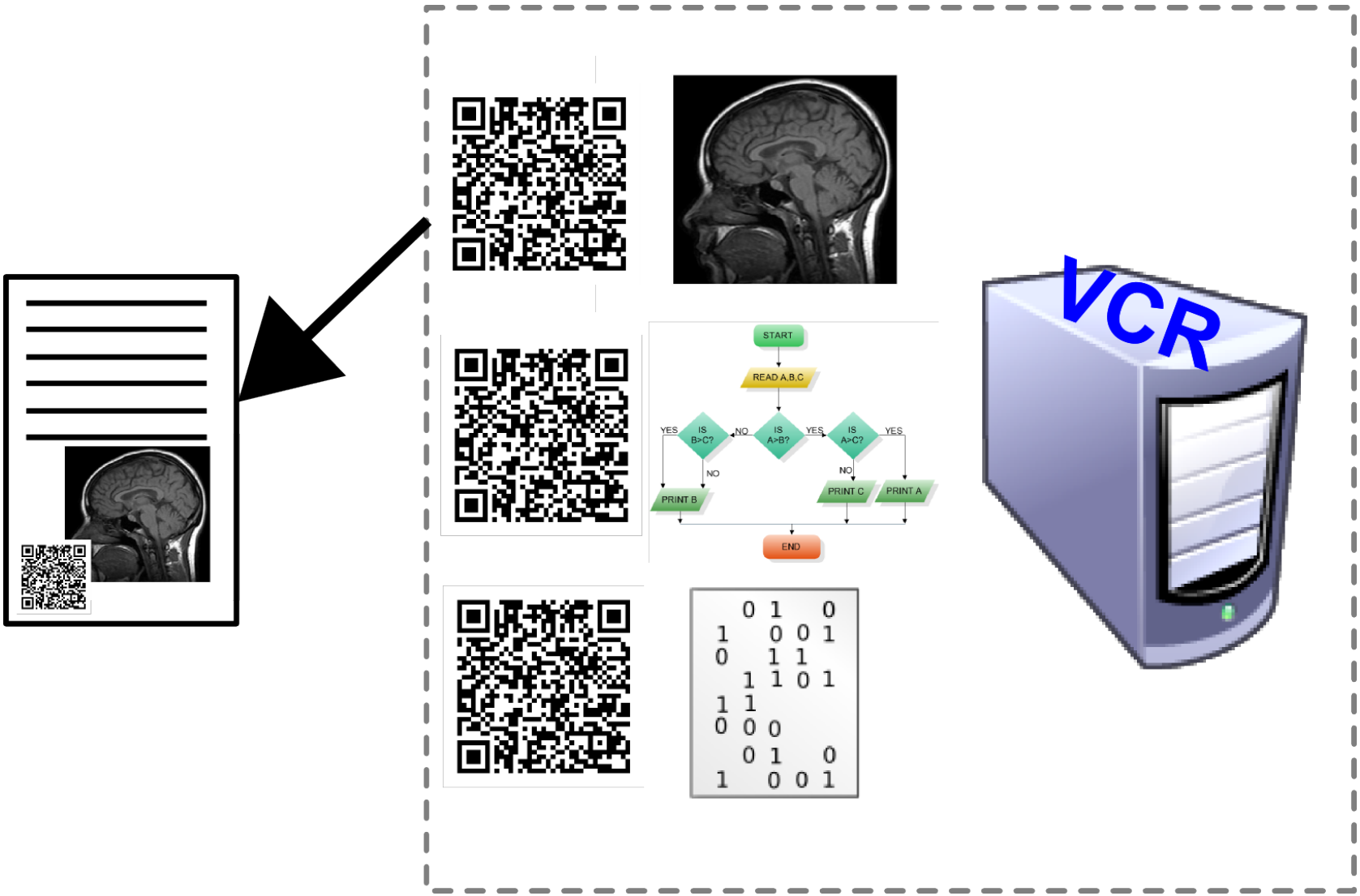

Simultaneous Structure and Texture Image Inpainting

Marcelo Bertalmio, Luminita Vese, Guillermo Sapiro, *Member, IEEE*, and Stanley Osher

***Abstract*—An algorithm for the simultaneous filling-in of texture and structure in regions of missing image information is presented in this paper. The basic idea is to first decompose the image into the sum of two functions with different basic characteristics, and then reconstruct each one of these functions separately with structure and texture filling-in algorithms. The first function used in the decomposition is of bounded variation, representing the underlying image structure, while the second function captures the texture and possible noise. The region of missing information in the bounded variation image is reconstructed using image inpainting algorithms, while the same region in the texture image is filled-in with texture synthesis techniques. The original image is then reconstructed adding back these two sub-images. The novel contribution of this paper is then in the combination of these three previously developed components, image decomposition with inpainting and texture synthesis, which permits the simultaneous use of filling-in algorithms that are suited for different image characteristics. Examples on real images show the advantages of this proposed approach.**

***Index Terms*—Bounded variation, filling-in, image decomposition, inpainting, structure, texture, texture synthesis.**

I. Introduction

THE filling-in of missing information is a very important topic in image processing, with applications including image coding and wireless image transmission (e.g., recovering

The algorithms reported in the literature best perform for pure texture, [10], [14], [28], or pure structure, [2], [3], [5]. This means that for ordinary images such as the one in Fig. 1, different techniques work better for different parts. In [26], it was shown how to automatically switch between the pure texture and pure structure filling-in process. This is done by analyzing the area surrounding the region to be filled-in (inspired by [17]), and selecting either a texture synthesis or a structure inpainting technique. Since most image areas are not pure texture or pure structure, this approach provides just a first attempt in the direction of simultaneous texture and structure filling-in (attempt which was found sufficient for the particular application of transmission and coding presented in the paper). It is the goal of this paper to advance in this direction and propose a new technique that will perform both texture synthesis and structure inpainting in all regions to be filled-in.

The basic idea of our algorithm is presented in Fig. 2, which shows a real result from our approach. The original image (first row, left) is first decomposed into the sum of two images, one capturing the basic image structure and one capturing the texture (and random noise), second row. This follows the recent work by Vese and Osher reported in [30], [31]. The first image is inpainted following the work by Bertalmio-Sapiro-Caselles-Ballester described in [5], while the second one is filled-in with a texture synthesis algorithm following the work by Efros and

Fig. 1. Example of image with both texture and structure.

decomposition, see Section V and [31]), to further enhance the results of the texture synthesis algorithm.[1]

II. Image Decomposition

In this section, we review the image decomposition approach proposed in [30], [31], which is one of the three key ingredients of the simultaneous texture and structure image inpainting

functions of bounded variation $\mathrm{BV}(\mathbb{R}^2)$, [12], allowing for edges

$$\inf_{u\in \mathrm{BV}} \left\{ F(u) = \int |\nabla u| + \lambda \|v\|_{L^2}^2, I = u + v \right\} \quad (1)$$

where $\lambda > 0$ is a tuning parameter. The second term in the energy is a fidelity term, while the first term is a regularizing term, to remove noise or small details, while keeping important features and sharp edges.

In [23], Meyer proved that for small λ the model will remove the texture. To extract both the $u \in \mathrm{BV}$ (a piecewise constant or cartoon representation of the image), and the v component as an oscillating function (texture or noise) from I; see Fig. 3, Meyer proposed the use of a different space of functions, which is in some sense the dual of the BV space (and therefore, contains oscillations). The idea is that if (1) (or wavelet-type decompositions) is used, then v will not just contain oscillations, but also undesired brightness edges. Meyer introduced the following definition, and also proved a number of results showing the explicit relationship between the $\|\ \|_*$ norm below and the model in [27] (see [23], [31] for details).

Definition 1: Let G denote the Banach space consisting of all generalized functions $v(x, y)$ which can be written as

$$v(x, y) = \partial_x g_1(x, y) + \partial_y g_2(x, y), \quad g_1, g_2 \in L^\infty(\mathbb{R}^2) \quad (2)$$

induced by the norm $\|I\|_*$ defined as the lower bound of all L^∞ norms of the functions $|g|$ where $g = (g_1, g_2)$, $|g(x, y)| = \sqrt{g_1(x, y)^2 + g_2(x, y)^2}$ and where the infimum is computed over all decompositions (2) of I.

Fig. 7. Object removal. The original image is shown on top-left, followed by the result of our algorithm (top-right) and the results with pure texture synthesis (bottom-left), failing to reconstruct the shoulder and introducing artifacts, and pure inpainting (bottom-right), giving a smoother reconstruction.

examples in this paper. The only parameters that vary are λ and the number of steps in inpainting, although the results were found to be very stable to these parameters as well.[2] Texture synthesis can be performed reasonably fast with the extensions in [13], [33], while image inpainting also takes just a few seconds. The overall algorithm takes about 2–3 minutes in a Pentium III, 800 MHz, without any optimization. Most of the

VI. Conclusions and Future Directions

In this paper, we have shown the combination of image decomposition with image inpainting and texture synthesis. The basic idea is to first decompose the image into the sum of two functions, one that can be efficiently reconstructed via inpainting and one that can be efficiently reconstructed

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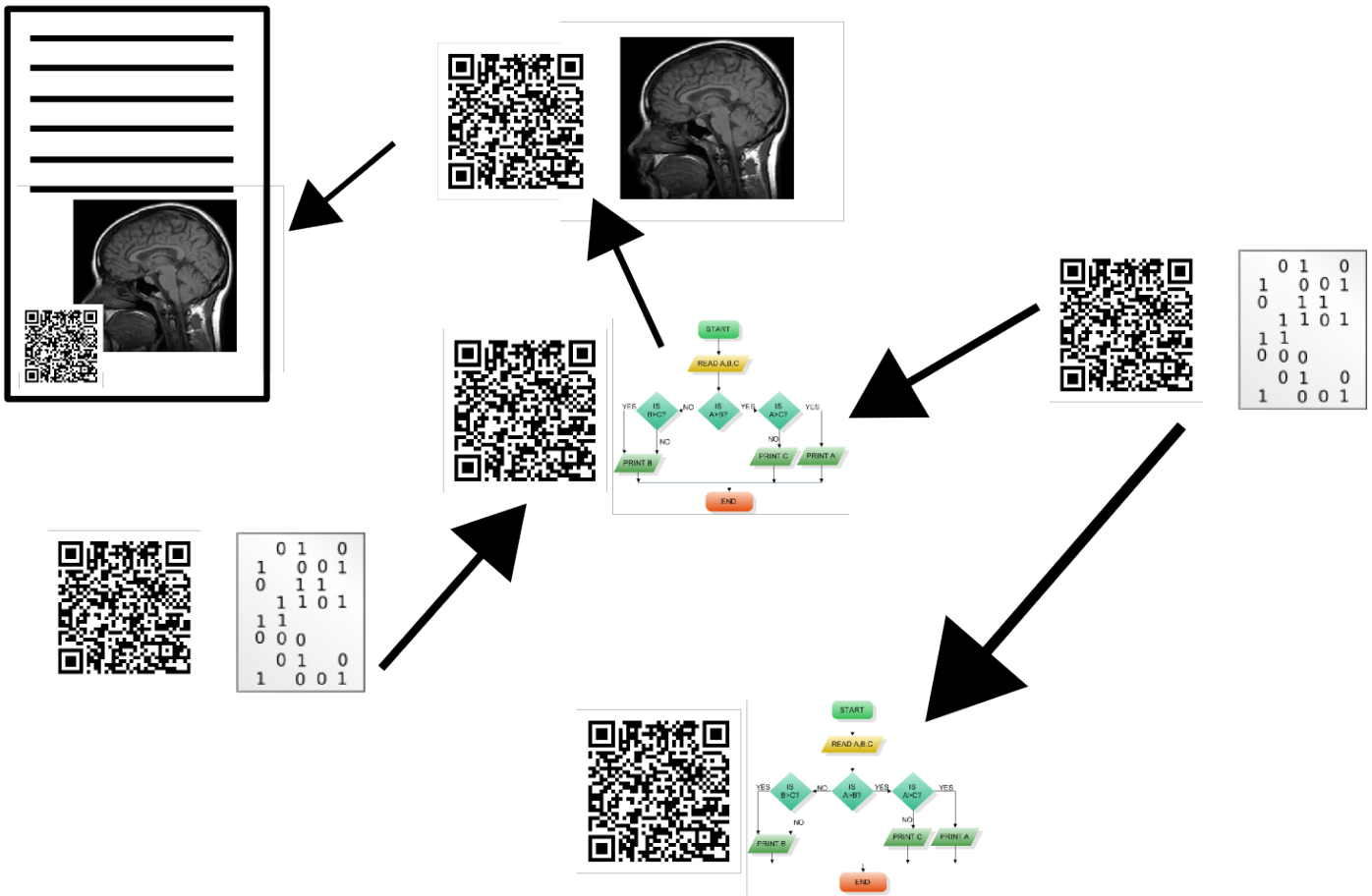
latex plugin

VRI's Everywhere!



Basic VCR apps

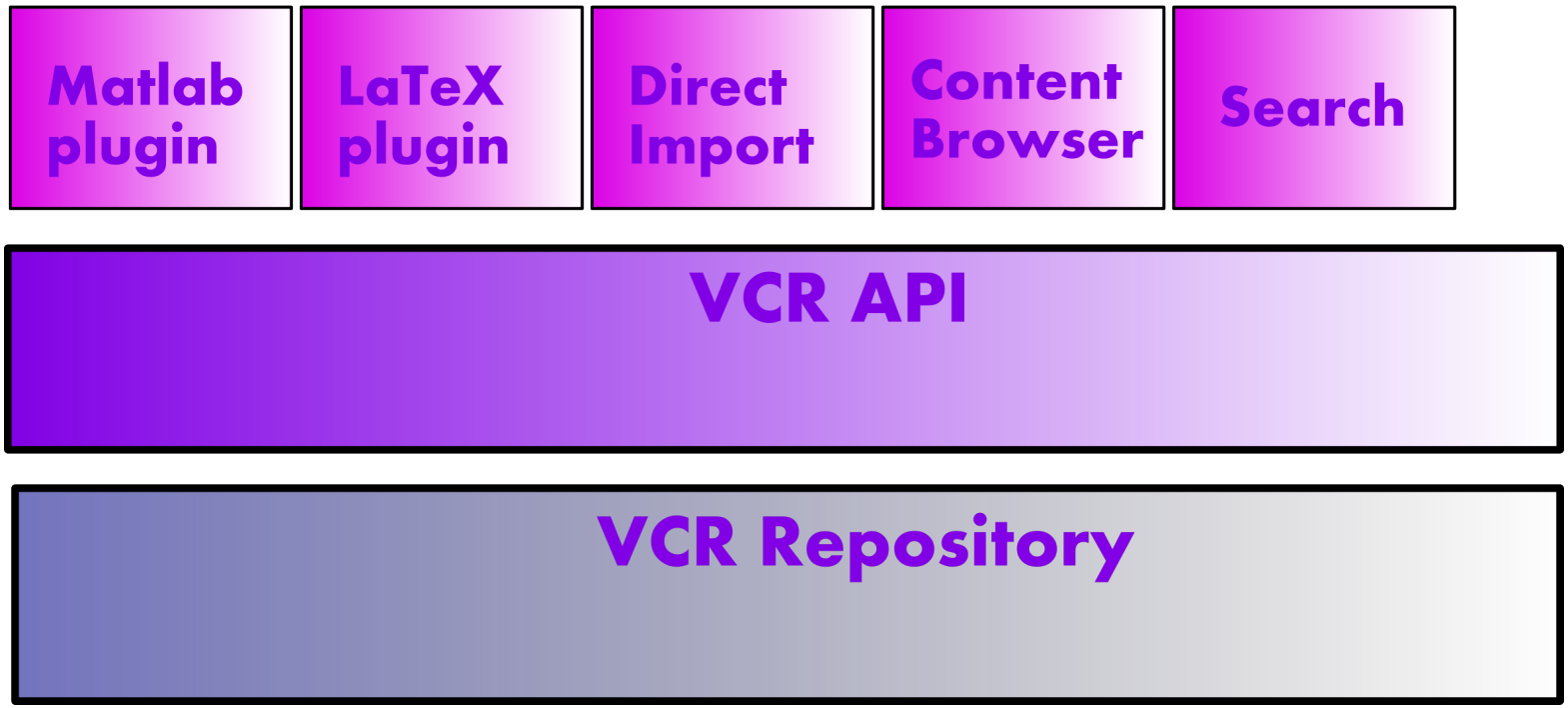
- Direct data import
- Word-processor plugin
- Content browser
- Search

Basic VCR apps elegantly solve pressing issues in science

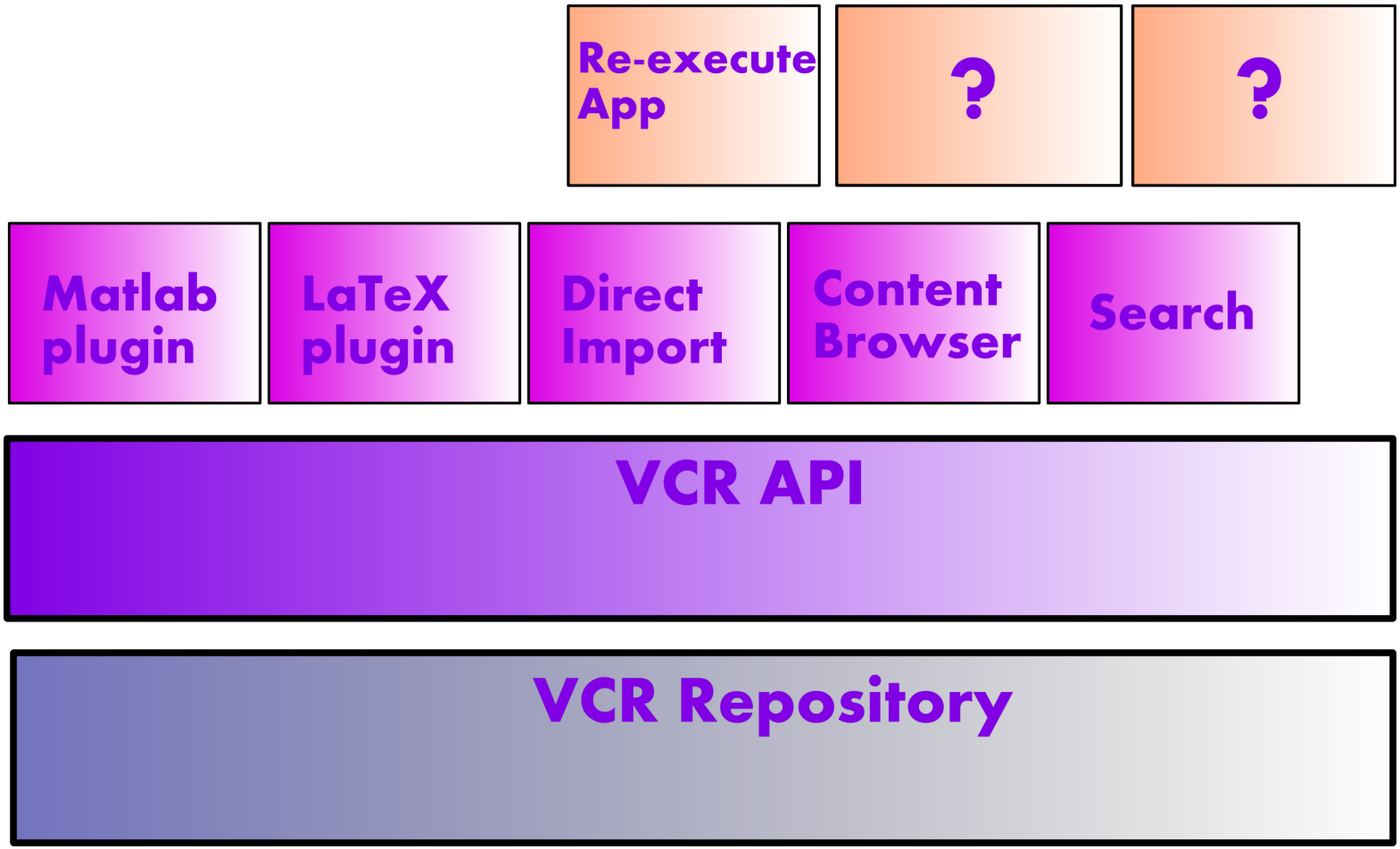
- Mandatory Data/code sharing policy
- Credibility concerns in computer science (e.g SIGGRAPH photoshopped results)
- Knowledge accumulation in research groups

Advanced VCR applications

Advanced VCR applications



Advanced VCR applications



Adopting the VCR foundation means:

For journals –

- Print VRI's next to results, cite data by VRI
- Operate VCR repository servers

For researchers/authors –

- Access all data and results through VRIs, not files
- Use VCR plugin that creates content and VRIs automatically during computation

For readers –

- Click/scan result VRI, browse underlying content

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For a solution to become standard, it must be –

- Technologically and legally realistic
- Easy for everyone to adopt today
- Offer real benefits for everyone

Adopting the VCR Foundation is technologically realistic

Technologically

- Publisher operates only repository server
- VCR repository based on secure, scalable Web 2.0 technology
- Applications do not run on public servers
- Readers need web browser - same paper/electronic article format

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The VCR Foundation is easy for everyone to adopt today

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Print URL + barcode: subtle change in print format

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For publishers

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- Stay in content validation, management and addressing business

For researchers/authors

- VCR applications – powerful research and knowledge discovery tools
- Group VCR repositories – productivity heaven
- Get explicitly cited for data

For readers

Uncover a rich content layer

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Uncover a rich content layer

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Microarray analysis of replicative senescence

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Background: Limited replicative capacity is a defining characteristic of most normal human cells and culminates in senescence, an arrested state in which cells remain viable but display an altered pattern of gene and protein expression. To survey widely the alterations in gene expression, we have developed a DNA microarray analysis system that contains genes previously reported to be involved in aging, as well as those involved in many of the major biochemical signaling pathways.

Results: Senescence-associated gene expression was assessed in three cell types: dermal fibroblasts, retinal pigment epithelial cells, and vascular endothelial cells. Fibroblasts demonstrated a strong inflammatory-type response, but shared limited overlap in senescent gene expression patterns with the other two cell types. The characteristics of the senescence response were highly cell-type specific. A comparison of early- and late-passage cells stimulated with serum showed specific deficits in the early and mid G1 response of senescent cells. Several genes that are constitutively overexpressed in senescent fibroblasts are regulated during the cell cycle in early-passage cells, suggesting that senescent cells are locked in an activated state that mimics the early remodeling phase of wound repair.

Conclusions: Replicative senescence triggers mRNA expression patterns that vary widely and cell lineage strongly influences these patterns. In fibroblasts, the senescent state mimics inflammatory wound repair processes and, as such, senescent cells may contribute to chronic wound pathologies.

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Received: **16 March 1999**
Revised: **2 July 1999**
Accepted: **15 July 1999**

Published: **23 August 1999**

Current Biology 1999, 9:939–945
http://biomednet.com/elecref/0960982200900939

0960-9822/99/$ – see front matter

Background

Historically, cellular senescence has been defined as the terminal phase of passaged primary human cell populations, a response more accurately defined as replicative senescence. It is now recognized, however, that a similar phenotype can be achieved in both normal and transformed cells by a variety of challenges, such as oxidative stress [1,2], radiation [3], activated oncoproteins and kinases [4–6], cyclin-dependent kinase (CDK) inhibitors [7] and others [8], leading to the concept that cellular senescence represents a fate choice that can be influenced by both pro- and anti-mitogenic stimuli. Senescent cells are not stimulated to divide by serum or passage in culture, and senescence invokes a specific cell-cycle understand the baseline changes in gene expression at senescence, we have used cDNA microarrays to study senescence-induced gene expression patterns in different cell types. The cell types examined here — dermal fibroblasts, retinal pigment epithelial cells and vascular endothelial cells — represent biologically diverse lineages that senesce with similar gross phenotypes. The invoked gene expression patterns differ greatly in a cell-type specific manner, however, suggesting that many of the observed alterations result from collateral activation of pathways that are set by cell lineage.

Results

Expression profiles of cell strains at replicative senescence

Figure 1

	BJ	RPE340	HUVEC
PDL	93.0	53.5	49.5
TRF (kbp)	6.3	4.3	6.1
%G1	71.4	81.8	84.5
%S	4.5	1.9	3.9
%G2	24.1	16.2	9.8

Current Biology

Analyses of senescent primary human cell lines. Photomicrographs are of senescent cultures stained for senescence-associated β-galactosidase (SA-βGal) [10]. Population doubling levels (PDLs) for senescent cultures are indicated, as well as telomere length (mean TRF in kilobase pairs (kbp)), and cell-cycle stage distribution as determined by multicycle FACS analysis.

RPE340 [14] and HUVEC (E.C., unpublished observations) can be immortalized by the expression of telomerase from an hTERT transgene. By these criteria, all three strains of cells undergo a similar arrest process — replicative senescence — initiated by a common effector, the critical shortening of telomeres.

Genes showing at least a 2.5-fold differential in expression in senescent and early-passage cells in multiple independent experiments are listed in Figure 2. Compared with quiescent early-passage cells, BJ fibroblasts at senescence have higher levels of the matrix-regulating proteins stromelysin-1 and stromelysin-2, plasminogen activator inhibitors PAI-1 and PAI-2, and urokinase plasminogen activator (uPA) as well as the inflammatory regulators monocyte chemotactic protein-1 (MCP-1), Gro-α, and interleukin-15 (IL-15). Conversely, senescent fibroblasts significantly underexpressed mRNAs for prostaglandin-1 synthase, elastin, stromelysin-3 and other proteins.

In high-serum conditions, genes that participate in cell division (for example those for cyclin A, cyclin B1, Cdc20, Cdc2, thymidine kinase and thymidylate synthase) are clearly expressed at higher levels in early-passage fibroblasts. Under these conditions, genes for secreted collagens Iα1 and IIIα1, elastin and cytokeratin II type 7 are also more highly expressed in early passage cells.

(Gas1) and growth-arrest and DNA-damage inducible protein 153 (GADD153). In addition, levels of mRNA for insulin-like growth factor binding proteins 2 and 5 (IGF-BP2, IGF-BP5), stanniocalcin and cathepsin O were elevated, and levels of peptidyl-α amidating monooxygenase (PAM), a prohormone-converting enzyme [16], remained high regardless of serum conditions.

The expression patterns observed in the other two cell types differed significantly from that of the dermal fibroblasts. In RPE340 cells, the differences in expression of mRNA for IGF-BP2, in both high and low serum, and a notable inability of the senescent cells to express a series of collagens in low-serum conditions were most obvious. Follistatin, a potential activin antagonist protein [17], was significantly expressed in senescent RPE340s whereas senescence repressed the expression of prostaglandin D synthase, a potential retinoid-binding protein [18], and cellular retinol-binding protein-1. Unlike BJ cells, senescence in RPE340s does not result in a significant increase in the expression of inflammatory chemokines or cytokines.

HUVEC cells display a dramatically different pattern of gene expression, in which many of the markers of senescence in fibroblasts are expressed in an inverse fashion. Inflammatory and immune-response genes such as those for IL-15 and Toll protein are all repressed in senescent HUVECs at low serum, as are the matrix proteases. Senescence in HUVECs induces the expression of IGF-BP5, neurofilament subunit L, transforming growth factor-β2 (TGFβ2) and adenosine A2A receptor, regardless of serum conditions. In high-serum conditions, ICAM-1 and GADD153 are overexpressed at senescence, as in fibroblasts.

To assess the extent that senescence-associated patterns of gene expression are preserved among cell strains of similar type, we examined two additional dermal fibroblast lines. C4 and MA were derived from the hand and ankle respectively of the same donor and are thus genetically identical. In general, patterns of gene expression in these two lines varied from that of BJ fibroblasts, though some shared trends are apparent. The expression of matrix proteases and inflammatory chemokines and cytokines at senescence is common to all the dermal fibroblast lines, though the set of expressed markers varies (Table 1a). A comparison of the MA and C4 strains suggests that the magnitude of the response at senescence and the particu-

Figure 2

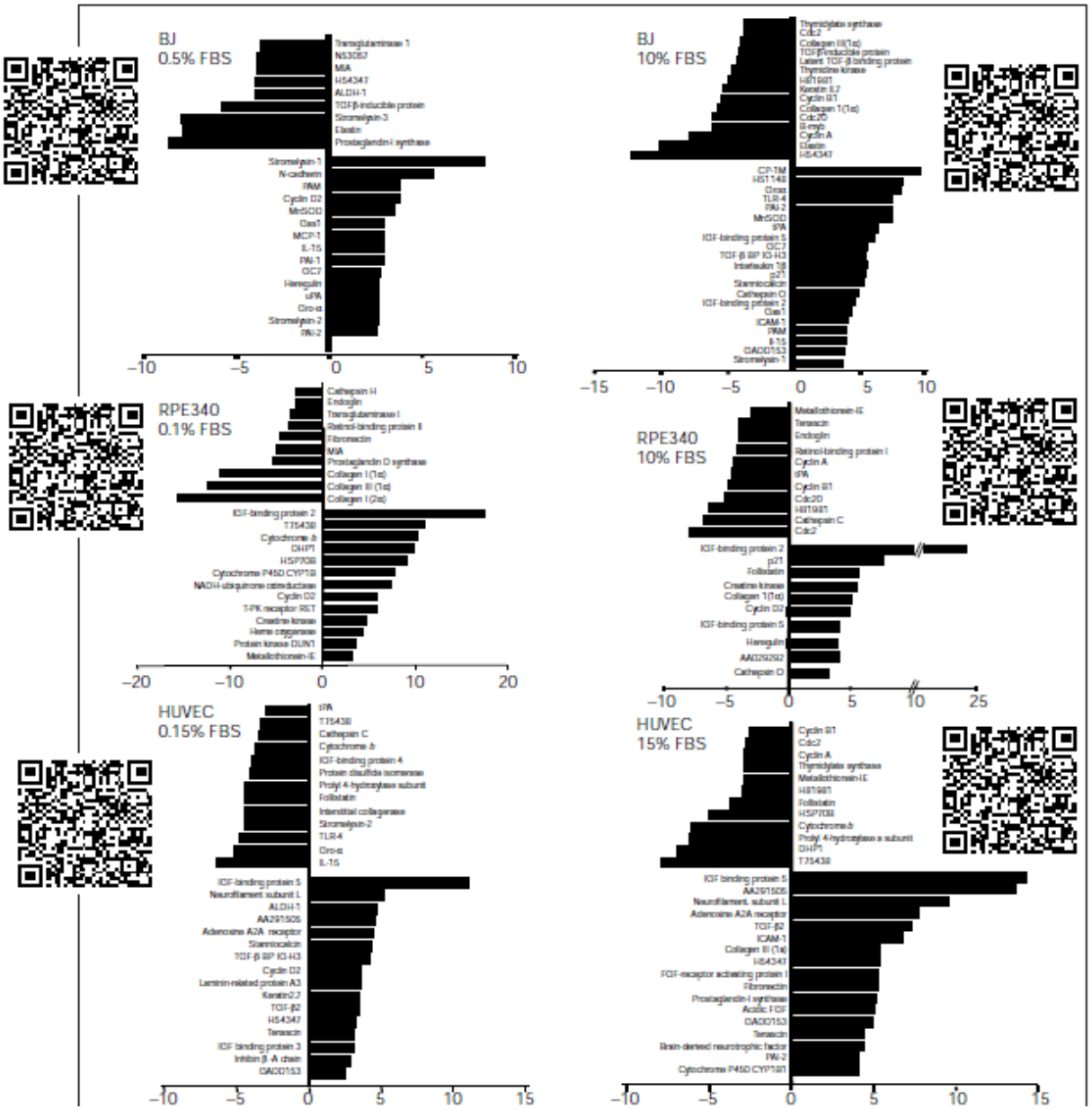

BJ
0.5% FBS
Transglutaminase 1
NS3DS7
MIA
HS4347
ALDH-1
TGFβ-inducible protein
Stromelysin-3
Elastin
Prostaglandin-I synthase
Stromelysin-1
N-cadherin
PAM
Cyclin D2
MnSOD
Cox1
MCP-1
IL-1S
PAI-1
GSC7
Hemogulin
uPA
Cox-α
Stromelysin-2
PAI-2
−10 −5 0 5 10
BJ
10% FBS
Thymidylate synthase
Cdc2
Collagen III(Tα)
TGFβ-inducible protein
Latent TGF-β binding protein
Thymidine kinase
H971697
Keratin 17
Cyclin B1
Collagen I(Tα)
Cdc20
B-myb
Cyclin A
Elastin
HS4347
CP-1M
H571448
Cmos
TLR-4
PAI-2
MnSOD
tPA
IGF-binding protein 5
GSC7
TGF-β BP IGI-H3
Interleukin 1β
p21
Stanniocalcin
Cathepsin D
IGF-binding protein 2
Cox1
ICAM-1
PAM
IL-1S
GADD153
Stromelysin-1
−15 −10 −5 0 5 10
RPE340
0.1% FBS
Cathepsin H
Endoglin
Transglutaminase I
Retinol-binding protein II
Fibronectin
MIA
Prostaglandin D synthase
Collagen I (Tα)
Collagen III (Tα)
Collagen I (2α)
IGF-binding protein 2
T75438
Cytochrome b
DHP1
HSP70B
Cytochrome P450 CYP1B
NADH-ubiquinone oxidoreductase
Cyclin D2
T-PK receptor RET
Creatine kinase
Heme oxygenase
Protein kinase DUN1
Metallothionein-IE
−20 −10 0 10 20
RPE340
10% FBS
Metallothionein-IE
Tenascin
Endoglin
Retinol-binding protein I
Cyclin A
tPA
Cyclin B1
Cdc20
H971681
Cathepsin C
Cdc2
IGF-binding protein 2
p21
Follistatin
Creatine kinase
Collagen I(Tα)
Cyclin D2
IGF-binding protein 5
Hemogulin
AA029292
Cathepsin D
−10 −5 0 5 10 25
HUVEC
0.15% FBS
tPA
T75438
Cathepsin C
Cytochrome b
IGF-binding protein 4
Protein disulfide isomerase
Prolyl 4-hydroxylase subunit
Follistatin
Interstitial collagenase
Stromelysin-2
TLR-4
Cox-α
IL-1S
IGF-binding protein 5
Neurofilament subunit L
ALDH-1
AA291505
Adenosine A2A receptor
Stanniocalcin
TGF-β BP IGI-H3
Cyclin D2
Laminin-related protein A3
Keratin2.7
TGF-β2
HS4347
Tenascin
IGF binding protein 3
Inhibin β -A chain
GADD153
−10 −5 0 5 10 15
HUVEC
15% FBS
Cyclin B1
Cdc2
Cyclin A
Thymidylate synthase
Metallothionein-IE
H971681
Follistatin
HS27038
Cytochrome b
Prolyl 4-hydroxylase α subunit
DHP1
T75438
IGF binding protein 5
AA291505
Neurofilament, subunit L
Adenosine A2A receptor
TGF-β2
ICAM-1
Collagen III (Tα)
HS4341
FGF-receptor activating protein 1
Fibronectin
Prostaglandin-I synthase
Acidic FGF
GADD153
Tenascin
Brain-derived neurotrophic factor
PAI-2
Cytochrome P450 CYP1B1
−10 −5 0 5 10 15

Table 1

(a) Pattern of gene expression in dermal fibroblasts at senescence.

	BJ		C4		MA	
%FBS	0.5	10	0.5	10	0.5	10
Stromelysin-1	8.2	4.1	1.0	22.0	–1.5	1.1
Stromelysin-2	2.6	2.0	1.6	5.9	1.9	1.6
Collagenase	2.0	1.7	–1.4	7.6	2.6	1.5
tPA	1.7	6.3	5.5	1.9	2.5	8.1
uPA	2.7	3.4	1.7	4.8	–1.2	1.2
MCP-1	2.9	9.0	2.7	4.3	2.5	3.4
Gro-α	2.8	6.6	1.6	5.8	2.5	1.2
IL-15	2.9	4.1	2.5	9.7	4.0	2.6
IL-1β	1.7	5.6	–1.2	3.1	1.3	1.2
Cathepsin O	2.9	4.8	4.2	3.9	2.2	4.2
Elastin	–8.4	–5.5	–4.5	–4.5	–8.3	–13.9
MnSOD	3.4	7.4	2.9	2.6	3.8	1.6
Tir-4	2.0	7.7	1.5	7.3	1.9	1.6
ICAM-1	–1.2	4.8	1.2	3.7	5.4	3.7

(b) Pattern of gene expression in retinal pigment epithelial cells at senescence.

	RPE340		RPE338		RPE341	
%FBS	0.1	10	0.1	10	0.1	10
IGF-binding protein-2	15.3	22.0	6.0	6.9	1.8	4.1
Collagen Iα2	–16.0	1.1	–10.6	–3.6	–58.0	–18.6
Collagen Iα1	–9.5	3.4	1.9	1.4	–26.5	–8.2
Collagen IIIα1	–9.8	1.4	1.9	3.3	–16.5	–4.0
Keratin I,18	1.4	–2.6	–3.8	–2.6	–8.1	–10.6
Keratin II,7	–1.0	–2.4	–18.5	–23.7	–24.8	–23.2
Transglutaminase 1	–3.5	–4.0	1.8	–1.9	–5.2	–3.0
Creatine kinase	3.6	7.3	3.9	2.3	1.6	2.8
Follistatin	–1.6	5.8	4.7	6.0	1.5	3.1
Cathepsin O	–2.1	3.0	2.5	2.7	24.3	11.7
Ceruloplasmin	1.4	1.7	1.0	3.7	14.6	7.0
Retinol-binding protein-1	–3.1	–3.2	1.2	1.2	–3.2	–4.4
Stanniocalcin	1.2	1.3	5.6	9.9	2.3	5.7
Thrombospondin 2	1.2	–3.5	–7.0	–3.3	–10.7	–16.8

RNA was prepared from three independent **(a)** dermal fibroblast or **(b)** RPE strains maintained in high or low serum. Positive numbers indicate fold-overexpression in senescent cultures; negative numbers indicate fold-overexpression in early passage cultures. See text and Supplementary material for abbreviations.

Time course of the response to serum stimulation

We compared the responses of early- and late-passage BJ fibroblasts to serum stimulation and a complete listing of responsive genes is provided as Supplementary material. In early-passage cells, late G1/early S-phase markers, such whereas in senescent cultures the response was attenuated (Figure 3a). Similarly, mRNAs for IκBα, tristetraproline (TTP), JunB and early growth response protein 1 (EGR-1) are all induced within 1 hour of stimulation in early-passage cells, whereas they are only weakly induced in senescent fibroblasts. The induction of mRNAs for connective tissue growth factor (CTGF) and mitogen-activated protein (MAP) kinase phosphatase 1 appeared unperturbed in senescent cells, indicating that substantial aspects of the early response to serum are maintained. Conversely, there was a pronounced induction of Id-2 in senescent cultures.

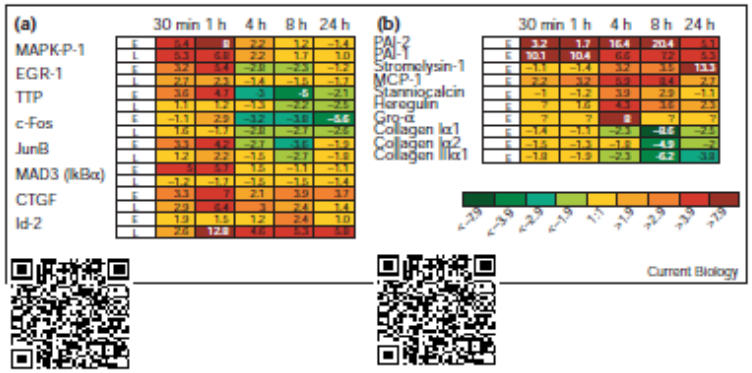
Several genes that are serum responsive in early-passage fibroblasts are constitutively upregulated in senescent cells. In early-passage cells, mRNAs for stromelysin-1, MCP-1, PAI-1, PAI-2 and others are induced by serum (Figure 3b). Conversely, collagen transcript levels decrease substantially following serum addition. The expression of many of the serum-inducible genes peaks near the 8 hour time point, at which time the cells would be in transit through the G1/S boundary.

Discussion

Senescence-associated gene expression

All three cell strains reported here undergo an arrest characterized by a distinctive cell-cycle distribution, changes in cell morphology and the shortening of telomeres. Senescent cells have a cell-cycle distribution that is clearly different from that of quiescent cells, as a substantial population of cells with G2 DNA content persists [9] (Figure 2). In high-serum conditions, this arrest includes expression of the CDK inhibitor p21, and growth arrest specific protein (Gas1) in BJ fibroblasts and RPE340 strains, but not in HUVECs. The overexpression of cyclin D2 was observed in all three cell strains and may reflect a common cell-cycle braking mechanism [19,20]. Interestingly, in none of the cell strains examined did we observe an upregulation of mRNA for the CDK inhibitor p16, as reported for other cell strains [21,22], and RT–PCR analysis of mRNA from senescent BJ fibroblasts confirmed the lack of induction of mRNA for p16 (data not shown). The induction of p21 at senescence has been reported to be transient in some cell lines, whereas the induction of p16 can occur after prolonged arrest [23], and this may also account for our observed results with different cell lines. The data presented here strongly suggest that even with a common initiating signal — shortened telomeres — the ensuing arrest may be triggered by dif-

Figure 3



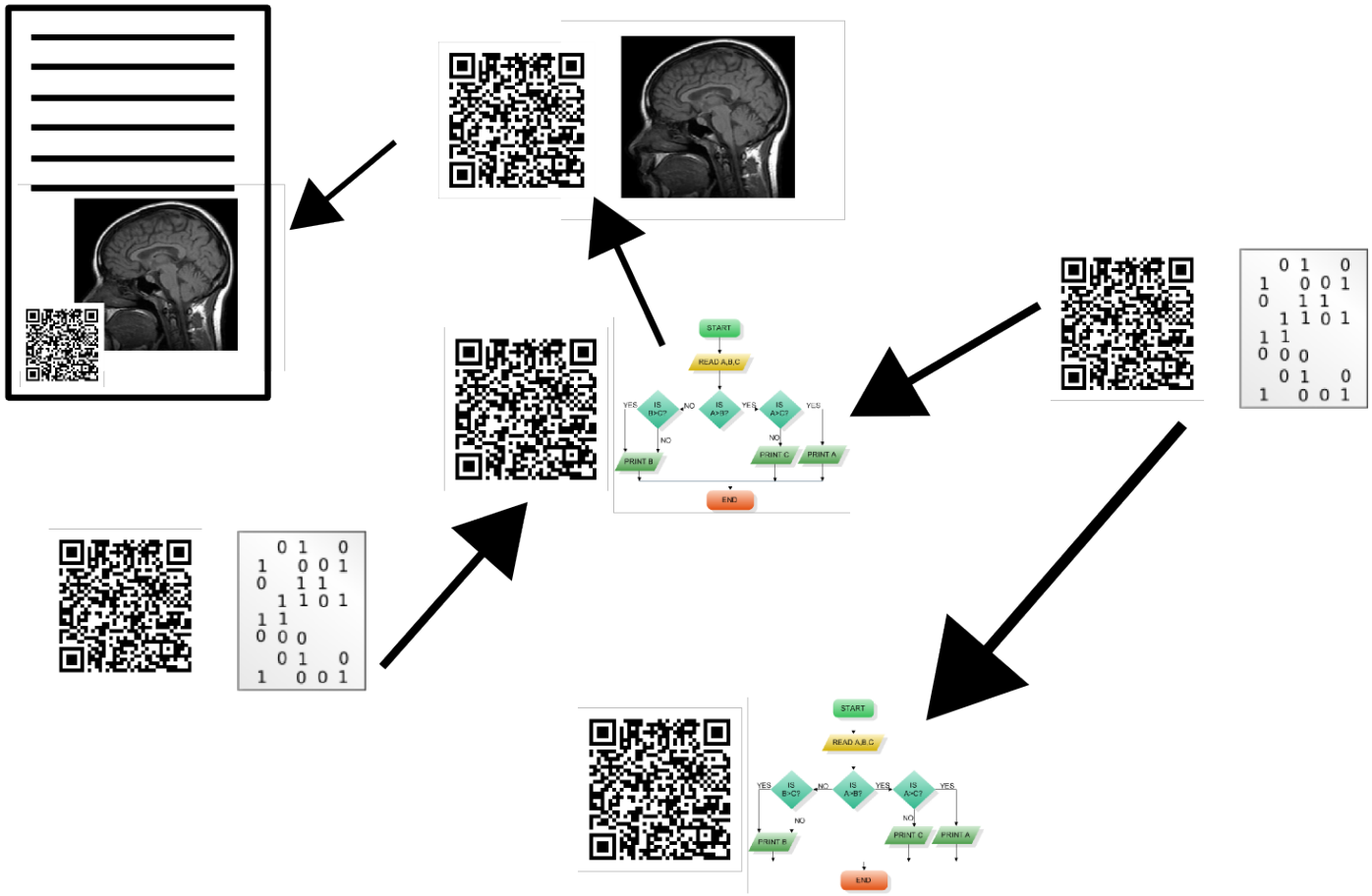
Time course of serum stimulation. **(a)** Early passage (E: PD30) or late passage (L: PD89) BJ cultures were held in 0.5% serum for 2 days, then stimulated with 10% FBS. RNA levels from cultures at the indicated time points (Cy5 channel) were compared with the uninduced starting culture (Cy3 channel). Positive values indicate higher expression in induced cells; negative values indicate lower expression in induced cells. Question marks indicate that there was insufficient signal for detection. A complete listing of serum-responsive genes from this analysis is provided in Supplementary material. **(b)** The serum-responsiveness of select senescence-regulated genes in early passage (PD30) BJ fibroblasts.

senescence response appears to overlap substantially with gene expression patterns observed in activated fibroblasts during wound healing [24–26]. MCP-1, Gro-α, IL-1β and IL-15 are strong effectors of macrophage and neutrophil recruitment and activation [27,28]. The upregulation of Toll (Tlr-4) in senescent fibroblasts confirms the overall immune response behavior at senescence. Tlr-4 is an IL-1 receptor homolog and is implicated in the activation of the gene regulatory protein NF-κB, a function proposed to be part of the innate immune response [29]. The induction of IL-15 at senescence is also consistent with an innate immune response, as IL-15 can be induced by NF-κB-dependent transcription [30] and also participates in inflammatory disease processes [28].

Deficiencies in the response of senescent cells to serum stimulation have been reported, and include an inability to induce the expression of c-*fos* mRNA [31] and markers of late G1 and S phase [32]. In response to serum, expression of inflammatory chemokines, matrix-degrading proteases and their modulators is induced in early-passage dermal fibroblasts, and expression of matrix collagens is reduced. This transient burst of activity may represent the natural response of these cells in wound repair [24]. Id-2 transcripts were hyper-induced in serum-stimulated senescent fibroblasts, which may be linked to the ability of cytokines to induce Id-2 expression [33]. The levels of mRNAs for many of these induced genes peak at a period predicted to span the G1/S boundary ([24] and this study); thus, the senescence response may mimic an activated state that states overlap substantially with those in telomere-induced senescence (W.F., D.N.S., R. Allsopp, S. Lowe, and G. Ferbeyre, unpublished observations) and thus are likely to use many of the same activation processes.

The pattern of gene expression at senescence varies substantially in different cell types. Although the expression of matrix and structural proteins, such as the collagens, keratins and auxiliary factors, is repressed in RPE cells, inflammatory regulators are not induced, in contrast to dermal fibroblasts. Physiologically, this would make sense, as an acute inflammatory response in a tissue critical for normal vision would be likely to have deleterious consequences. However, as the RPE layer has a central role in the deposition and maintenance of extracellular matrix in the retina, decrements in the ability of senescent RPE cells to maintain appropriate expression patterns, as evidenced by decreased expression of collagens, keratins, aggrecan, transglutaminase and so on, would be predicted to have adverse affects on retinal architecture. Dysfunction of the RPE cell layer is considered to be a substantial factor in the development of age-related macular degeneration [36].

Surprisingly, early-passage HUVECs overexpress many of the markers associated with senescence in dermal fibroblasts, such the pro-inflammatory molecules IL-15 and Tlr-4. In very low serum, vascular endothelial cells are often induced to undergo apoptosis, and the conditions used in this study (0.15% fetal bovine serum (FBS)) have been set to induce cessation of growth (quiescence) while


START
READ A,B,C
IS B>C?
IS A>B?
IS A>C?
PRINT B
PRINT C
PRINT A
END

Conclusion

16+ years of reproducible research, still no standard solution

We propose change of focus: automatic computation chronicling

VCR is ready now, shows potential of becoming standard

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Thank you.

Acknowledgments

Balasubramanian Narasimhan (Stanford), Alon Shalita (Facebook)

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