The Group I Intron Conspiracy
(another adventure in conceptual biology)

Literature and data suggest a functional origin of self-splicing introns in oxygenic photosynthesis

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Outline

- T4 phage *td* intron: a possible feedback regulation
- Light/redox regulated splicing of the *psbA* intron
- Self-splicing introns in protein-coding genes
- Genetic exchange in cyanobacterial phytoplankton
- Oxidative metabolism and self-splicing introns?
- How to proceed?
A mobile group I self-splicing intron in the T4 \( td \) gene

- \( td \) gene encodes for the dTMP Synthetase: \( dUMP \rightarrow dTMP \)
- \( nrdB \) and \( nrdD \) genes also contain group I introns
- Introns contain a homing endonuclease ORF: \( intron^- \rightarrow intron^+ \)

Miller et al. 2003 Microbiol Mol Biol Rev
Chu et al. 1984 PNAS, Gott et al. 1986 Cell
Waldsich et al. 2002 EMBO J
Mobile intron life cycle

HEG: homing endonuclease gene

highly conserved target sites: often adjacent to anticodon loops or catalytic centers

Belfort and Roberts 1997 NAR
Bell-Pedersen et al. 1990 NAR
Sandegren and Sjöberg 2004 JBC
T4 life cycle: Recombination Dependent Replication

Transcription

T4
Promoters: Early

Replication & Recombination

origin initiation

Single-Strand-Annealing Pathway I

Join-Copy Pathway II

Cut & paste or cut & replicate or cut & package

replicate cut & package

Packaging

Early RNA (term)

Core RNA Polymerase σ70
GTTTAC(17) gtggTAtaAT

Early RNA (rt)

Core RNA Polymerase σ70
HTTTTT(13) TAtaAT

Middle RNA

Late RNA

Late Proteins

Endonuclease VII

Terminase

Primase present

Primase absent

Late Proteins

Endonuclease VII

Terminase

Primase present

Primase absent

a perfect environment for HEG/intron mobility

Mosig 1998 Ann Rev Genet
PreRNA → mRNA → dTMP Synthetase

Positive splice regulators
- guanosines
- specific splicing factor Cyt-18 (Tyrosyl-tRNA synthetase)
- RNA chaperones, e.g. StpA
- HEG derived maturases

Negative splice regulators
- competitive, guanosine analog
  - deoxyguanosine and dideoxyguanosine
  - amino acid arginine
  - coenzyme flavin FMN
  - coenzyme thiamin pyrophosphates
  - antibiotics streptomycin, viomycin, capreomycin
- competitive, no guanosine analog
  - antibiotic lysinomycin
- non-competitive
  - aminoglycoside antibiotics
    - kanamycin, tobramycin, tetracycline, pentamidine, spectinomycin
  - NAD$^+$ > NADH
  - NADP$^+$ > NADPH


$td \text{ preRNA} \rightarrow td \text{ mRNA} \rightarrow \text{dTMP Synthetase}$

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    - $\text{NAD}^+ > \text{NADH}$
    - $\text{NADP}^+ > \text{NADPH}$

Park and Kim 2001 Biochem Biophys Res Comm
Kim and Park 2003 Mol Cell Biochem
$td \text{ preRNA} \rightarrow td \text{ mRNA} \rightarrow d\text{TMP Simthetase}$

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    - $NAD^+ \rightarrow NADH$
    - $NADP^+ \rightarrow NADPH$

Machné 2006, unpublished
Some interesting facts:

- *td* translation is required for splicing (Waldsich et al. 1998 RNA)
- E. coli’s dTMP synthetase binds its own mRNA (Voeller et al. 1995 NAR)
"When lightning strikes twice" (©Marlene Belfort) ...

Some interesting facts:

- **aerobic RNR (nrdA/B)** uses T4’s own glutaredoxins (*nrdC* and/or *nrdH*) as electron donors
- T4 glutaredoxin can be used in both, glutaredoxin and thioredoxin pathways (Holmgren 1978 JBC)
- **pH** dependence of *nrdB* splicing, low **pH** (up to 7) delays splicing (Sjögren 1997 NAR)
- **anaerobic RNR (nrdD/G)** uses formate and NTPs as substrates (Andersson et al. 2000 JBC)
- anaerobic RNR (nrdD/G) has not used UTP as a substrate *in vitro* (Andersson et al. 2000 JBC)
- introns in different subunits (small: *nrdB*, large: *nrdD*): **are mixed protein complexes (NrdAG)** feasible?
**nrdA/B, nrdD/G: the T4 aerobic and anaerobic RNRs**

“When lightning strikes twice” (©Marlene Belfort) ...

Note the different substrates!

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... well, at least 2 out 3 possible strikes.
T4 nucleotide metabolism: 50% → 35% GC

C. K. Mathews: Metabolite Channeling

5 reductive steps from RNA to DNA: 1/dNTP + 1/dTTP

T4 (168903 bp, 32.5% T), under aerobic conditions:
2.65 * 168903 = **447593** NADPH / phage DNA equivalent
(- 2 * 4.639.675 E. coli DNA, - ??? dT Ribose)
Group I introns and oxidative metabolism, so far:

- *td* intron: *in vitro* non-competitive splice inhibition by NADP⁺, a by-product of reactions catalyzed by the *frd* and *td* gene products.

- All of T4’s intron containing gene products are involved in the reductive steps of nucleotide metabolism.
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⇒ any examples for a redox regulation of group I splicing?
The *psbA* intron splicing is light/redox regulated

*Light-regulated splicing of psbA pre-RNAs*

The *Chlamydomonas reinhardtii* chloroplast *psbA* gene contains four large group I introns that self-splice efficiently in vitro, but only under nonphysiological conditions. These data suggest strongly that light coordinately stimulates splicing of all four *psbA* introns. Moreover, they demonstrate that this response to light is mediated by photosynthetic electron transport. Desphande et al. 1997 RNA

... This phenomenon suggests that at least one component required for [Neurospora] mitochondrial[25S] RNA splicing is regulated such that its synthesis or activity is increased in response to impairment of electron transport. Bertrand et al. 1982 Cell

Note the opposite effects of the two electron transport chains on splicing.
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Expression of psbA genes is regulated at multiple levels in the cyanobacterium Synechococcus sp. PCC 7942:

... in conditions where production of reduced Fd is in excess with respect to its consumption in carbon fixation (e.g. at high light or low temperature), the enzyme FdTR reduces Trx in significant amounts ...

... the split-tailed arrow represents a yet unknown mechanism by which the changes in the thiol redox state induce differential transcription [and translation] of the psbA genes. ...

Sippola and Aro 2000, Photochem Photobiol

Right: D1 repair cycle in photoinhibition

Cyanophage infection and photoinhibition in marine cyanobacteria. Bailey et al. 2004 Res Microbiol
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Cyanophages

podophages (T7-like) and myophages (T4-like)

Fuhrman 2003 Nature
Organization of the \textit{psbDA} genes in cyanophages

\begin{enumerate}
\item[8] T7-like head-to-tail connector;
\item[9] T7-like capsid assembly protein;
\item[10] T7-like capsid protein
\end{enumerate}

\textit{hli}: thought to protect the photosynthetic apparatus from excess excitation energy during stress

\begin{enumerate}
\item[\textit{petE}]: plastocyanin;
\item[\textit{petF}]: ferredoxin
\end{enumerate}

\begin{enumerate}
\item[nrdB]: T4-like ribonucleotide reductase -subunit
\item[49]: T4-like restriction endonuclease VII
\item[\textit{td}]: T4-like thymidylate synthase (P-SSM4: \textit{nrdC} between \textit{psbD} and \textit{td} !)
\end{enumerate}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{psbDA_genes.pdf}
\end{figure}

\textbf{NOTE}: black: host genes, white: unknown ORFs; grey: phage genes
Organization of the *psbDA* genes in cyanophages

- **intron**: group I, self-splicing
- **ORF178**: T4-like restriction endonuclease VII domain: involved in intron motility?
- **psbDA**: keep the host’s photosystem running, by supplying the D1/D2 repair cycle
- **S-PM2**: *nrdC* and *td* are 2.8 kb and 6.1 kb upstream

Mann et al. 2003 *Nature*, Millard et al. 2004 *PNAS*,
Organization of the *psbDA* genes in cyanophages

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*a light/redox regulated psbA intron could allow a simple PS adaptation in different hosts and/or environments*

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A light/redox regulated *psbA* intron could allow a simple PS adaptation in different hosts and/or environments


Phage *psbDA*: provide NADPH for RNA → DNA reduction?
‘Photosynthetic’ cyanophages
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- 1 example of each, light/redox and electron transport regulation of group I intron splicing.

- T4-like cyanophages carry a potentially regulatory group I intron in variable regions in the vicinity of *td* and *nrdB* genes.
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⇒ let’s take a broader look at group I introns!
Distribution of group I introns

- June 8 2004: 1400 group I introns
- 90% in fungi, plants, red and green algae
- Rare in bacteria and their phages, absent in archaea
- Animals: 2 in the mitochondrial genome of a sea anemone and 1 in the mitochondrial gene \textit{cytochrome c oxidase subunit I} of a coral
- Nucleus: 800 at 47 different sites in SSU rRNA and 44 sites in LSU rRNA \Rightarrow only rRNA
- 220 in mitochondrial genes and 370 are in plastid DNA \Rightarrow rRNA, tRNA and proteins

Haugen et al. 2005 Trends in Genetics
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Haugen et al. 2005 Trends in Genetics
### Group I introns in protein coding genes, eukar.

<table>
<thead>
<tr>
<th>Introns</th>
<th>Gene (ID)</th>
<th>Protein</th>
<th>Function</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>aI5α</td>
<td>coxl (div.)</td>
<td>cytochrome c oxidase</td>
<td>Respiration</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>I</td>
<td>cob (div.)</td>
<td>cytochrome b</td>
<td>&quot;</td>
<td>Ascomycete P. a.</td>
</tr>
<tr>
<td>I</td>
<td>nad5 (P26849)</td>
<td>NADH dehydrog. SU5</td>
<td>&quot;</td>
<td>Liverwort M.p.</td>
</tr>
<tr>
<td>IA1</td>
<td>psaB (P36492)</td>
<td>P700 chlorophyll a apoprotein A2</td>
<td>PS I</td>
<td>Chlamydomonas m.</td>
</tr>
<tr>
<td>IB4, IA1</td>
<td>psbC (Q08684)</td>
<td>P6 protein</td>
<td>PS II</td>
<td>&quot;</td>
</tr>
<tr>
<td>IA1</td>
<td>psbA (X13486)</td>
<td>D1 protein</td>
<td>PS II</td>
<td>Chlorophyceae</td>
</tr>
<tr>
<td>IA1</td>
<td>rbcL (BAC06369)</td>
<td>Rubisco large SU (carboxylase)</td>
<td>calvin cycle</td>
<td>Chlorella vulgaris C-27</td>
</tr>
<tr>
<td>I</td>
<td>chlL (P56291)</td>
<td>protochlorophyllide reductase</td>
<td>chlorophyll synth.</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>div.</td>
<td>URF14.2</td>
<td>homospermidine syn.</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>div.</td>
<td>TFIIS</td>
<td>transcription</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>div.</td>
<td>capsid Vp52</td>
<td>major capsid protein</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
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<tbody>
<tr>
<td>I + ins.el.</td>
<td>tcdA-C34</td>
<td>enterotoxin</td>
<td>causes diarrhoea</td>
<td>Clostr. difficile</td>
</tr>
<tr>
<td>I</td>
<td>recA (AAK00736)</td>
<td>RecA DNA recombination</td>
<td>SOS DNA repair</td>
<td>Geobac. kaustophilus</td>
</tr>
<tr>
<td>IA2</td>
<td>polAc (AY769989)</td>
<td>DNA Pol A domain</td>
<td>DNA synth.</td>
<td>T7-like W31/Phi I</td>
</tr>
<tr>
<td>IA1/intein</td>
<td>bnrdE/bnrdF</td>
<td>aerobic RNR LSU</td>
<td>dNTP synth.</td>
<td>Bacillus SPβ proph.</td>
</tr>
<tr>
<td>2 * I</td>
<td>nrdE</td>
<td>“</td>
<td>“</td>
<td>Twort (Staph. aureus)</td>
</tr>
<tr>
<td>3 * I</td>
<td>orf142</td>
<td>late gene product</td>
<td>tail sheath protein ?</td>
<td>“</td>
</tr>
<tr>
<td>IA2</td>
<td>nrdB (NP_049841)</td>
<td>aerobic RNR SSU</td>
<td>dNTP synth.</td>
<td>T4 coliphages</td>
</tr>
<tr>
<td>IA2</td>
<td>nrdD (NP_049690)</td>
<td>anaerobic RNR LSU</td>
<td>“</td>
<td>“</td>
</tr>
<tr>
<td>IA2</td>
<td>td (NP_049848)</td>
<td>dTMP synth.</td>
<td>“</td>
<td>“</td>
</tr>
<tr>
<td>I</td>
<td>psbA</td>
<td>D1 protein</td>
<td>PSII</td>
<td>Cyanophages</td>
</tr>
<tr>
<td>2 * I</td>
<td>terL (AAR27298)</td>
<td>terminase LSU</td>
<td>DNA cleavage</td>
<td>Lactobac. phage</td>
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<tr>
<td>I</td>
<td>endo. (AAF24750)</td>
<td>endonuclease</td>
<td>“</td>
<td>Phages of</td>
</tr>
<tr>
<td>IA2</td>
<td>lysin (AAF24749)</td>
<td>lysozyme (S-S formation)</td>
<td>lysis</td>
<td>Strept. thermophilus</td>
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</tbody>
</table>
### Group II introns in protein coding genes

<table>
<thead>
<tr>
<th>Introns</th>
<th>Gene (ID)</th>
<th>Protein</th>
<th>Function</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>nad4 (P26848)</td>
<td>NADH dehydrog. SU4</td>
<td>Respiration</td>
<td>Liverwort M.p.</td>
</tr>
<tr>
<td>II</td>
<td>nad2 (P26846)</td>
<td>NADH dehydrog. SU2</td>
<td>“</td>
<td>Wheat T.aestivum</td>
</tr>
<tr>
<td>II</td>
<td>nad7</td>
<td>NADH dehydrog. SU7</td>
<td>“</td>
<td>“</td>
</tr>
<tr>
<td>4 * II</td>
<td>nad2 (3800093)</td>
<td>NADH dehydrog. SU2</td>
<td>“</td>
<td>Rice</td>
</tr>
<tr>
<td>3 * II</td>
<td>nad4 (X57164)</td>
<td>NADH dehydrog. SU4</td>
<td>“</td>
<td>Hordeum vulgare</td>
</tr>
<tr>
<td>II</td>
<td>rpl2</td>
<td>mitoch. ribosomal pr. L2</td>
<td>“</td>
<td>Mustard (S. alba L.)</td>
</tr>
<tr>
<td>II</td>
<td>ndhA (4150866)</td>
<td>NADH dehydrog.</td>
<td>“</td>
<td>Maize, Spinach</td>
</tr>
<tr>
<td>2 * II</td>
<td>ycf3-psaAB</td>
<td>Yfc3</td>
<td>“</td>
<td>Euglena gracilis</td>
</tr>
<tr>
<td>II</td>
<td>atpF</td>
<td>ATPase SU F</td>
<td>“</td>
<td>“</td>
</tr>
<tr>
<td>9 * II</td>
<td>psaA, psaB</td>
<td>P700 chlorophyll a apoproteins</td>
<td>Photosystem</td>
<td>“</td>
</tr>
<tr>
<td>II, w. ORF mat2</td>
<td>psbC (P05700)</td>
<td>P6 protein</td>
<td>Photosystem</td>
<td>“</td>
</tr>
<tr>
<td>3 * II in mat2</td>
<td>mat2 (P05728)</td>
<td>Maturase</td>
<td>Photosys. ATPase</td>
<td>“</td>
</tr>
<tr>
<td>2 * III, psbCi4</td>
<td>psbC (P05700)</td>
<td>P6 protein</td>
<td>PS I</td>
<td>“</td>
</tr>
<tr>
<td>9 introns</td>
<td>atpI/H/F/A,</td>
<td>ATPase</td>
<td>PS II</td>
<td>“</td>
</tr>
<tr>
<td>“ (operon)</td>
<td>rps2/18</td>
<td>ribosomal pr. S2/S18</td>
<td>intron splicing</td>
<td>“</td>
</tr>
<tr>
<td>II twintron</td>
<td>psbF (CAA77913)</td>
<td>β-subunit of cytochrome b-559</td>
<td>PS II</td>
<td>“</td>
</tr>
<tr>
<td>54 * III</td>
<td>div.</td>
<td>ribosomal pr.</td>
<td>ribosomal</td>
<td>“</td>
</tr>
<tr>
<td>II (from cyanob)</td>
<td>psbA (AAQ84047)</td>
<td>D1 protein</td>
<td>PS cyt b/f</td>
<td>“</td>
</tr>
<tr>
<td>IIIB2</td>
<td>psbA (AY325305)</td>
<td>D1 protein</td>
<td>transcr./transl.</td>
<td>“</td>
</tr>
<tr>
<td>II</td>
<td>recA</td>
<td>RecA DNA recombination</td>
<td>PS II</td>
<td>E. myxocylindracea</td>
</tr>
<tr>
<td>4 inteins + 3 II</td>
<td></td>
<td>RNR</td>
<td>SOS DNA repair</td>
<td>Chlam. sp.CCMP 1619</td>
</tr>
<tr>
<td>“</td>
<td>nrd</td>
<td></td>
<td>dNTP synth.</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>“</td>
<td>nrd</td>
<td></td>
<td>“</td>
<td>cyanobacterium</td>
</tr>
<tr>
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<td>Trichodesmium e.</td>
</tr>
</tbody>
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⇒ let’s take a look at the phytoplankton ecosystem!
Photosynthesis: gaia’s entropy reducer

Phytoplankton and the cyanobacteria ‘metaspecies’:

► 50 % of world’s $O_2$ production
► Origin and maintenance of the $O_2$ world
  ⇒ invention of oxygenic photosynthesis and oxidative respiration
► Different species at different marine strata:
  ► high-light, few nutrients
  ► adaptive (e.g. different psbA versions)
  ► low-light, more nutrients
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► Genomic exchange, e.g. between cyanobacteria and their phages

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Evolution of the photosystems ... 

(... and of oxidative phosphorylation!)

Olson and Blankenship 2004 Photosynthesis Res
Xiong and Bauer 2002 Annu Rev Plant Biol
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Could mobile introns with splicing sensitivity to redox state spread a simple adaptation mechanism, e.g. during transition from the anaerobic to the aerobic ($O_2$) world?
Mobile introns of rRNA and tRNA?

- Positive td splice regulators
  - guanosines
  - specific splicing factor Cyt-18 (Tyrosyl-tRNA synthetase)
  - RNA chaperones, e.g. StpA
  - HEG derived maturases

- Negative td splice regulators
  - competitive, guanosine analog
    - deoxyguanosine and dideoxyguanosine
    - amino acid arginine
    - coenzyme flavin FMN
    - coenzyme thiamin pyrophosphates
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    - $\text{NAD}^+ > \text{NADH}$
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- Balance replication / transcription / translation with redox conditions?
- Emergency switch for cellular redox reactors?
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