

Presence of different HPV 16 RNA transcripts in normal cells compared to CIN2, CIN3 and SCC biopsies

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Objective

To investigate viral transcript patterns in HPV 16 DNA positive normal Pap smears and cervical intraepithelial neoplasia (CIN) grade 2, 3 and cervical squamous cell carcinoma (SCC) biopsies.

Methods

HPV 16 transcripts were detected using four primer-sets (Figure 1) and nucleic acid sequence based amplification (NASBA) on CIN2 (n=7), CIN3 (n=21), SCCs (n=116) and normal cervical cell samples (n=75). NASBA is poly-A independent and amplifies single-stranded RNA directly, even in a DNA background (Figure 2, 3, 4). HPV 16 DNA was detected by PCR while the physical state of the viral genome in SCCs was examined by *in situ* hybridization (ISH) (Figure 5). The biopsies were fixed in formalin and embedded in paraffin, while the normal cell samples were dispersed in NucliSens lysis buffer (bioMérieux, Marcy l'Etoile, France).

Results

Viral transcripts were detected in 100% of the histological SCCs in contrast to 77% of HPV 16 DNA positive normal cells. The detection frequencies of the different primer-sets are shown in table 1 and 2.

Discussion

Frequent viral transcription was identified in CIN2, CIN3 and SCCs, especially of the E6 and E7 oncogenes, in contrast to HPV 16 DNA positive normal cells where approximately 1/4 lack any viral transcription. The E6/E7 full-length transcript was detected in all CIN2, CIN3 and SCC biopsies and in 1/3 of the normal cells. In addition, the E6*/I/II transcripts were detected in nearly all the CIN2, CIN3 and SCC biopsies and in about 3/5 of the cytologically normal smears indicating a lower clinical specificity than detection of E6/E7 full-length transcript. The E1^ΔE4^ΔL1 and E1^ΔE4E5 transcripts were detected in roughly half the SCCs with only integrated viral DNA as compared to SCCs with integrated as well as episomal DNA. This supports the current understanding about disruption within the E2 gene region upon viral integration in SCCs. However, we cannot be sure that these samples are not containing small amounts of episomal DNA as well, due to limitations of the ISH method.

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Table 1. Detection of HPV 16 transcripts in normal cells and CIN2, CIN3 and SCC lesions

Diagnose	E6/E7	Transcripts		
		E6*/I/II	E1 ^Δ E4 ^Δ L1	E1 ^Δ E4E5 ^a
Normal ^a (n=75)	35%	61%	41%	60%
CIN2 ^b (n=7)	100%	86%	43%	86%
CIN3 (n=21)	100%	95%	33%	76%
SCC (n=116)	100%	98%	53%	76%

^a The normal diagnoses refer to cytology. ^b The CIN2, CIN3 and SCC diagnoses refer to histology. ^c The E1^ΔE4E5 primer-set also detect the E6E7E1^ΔE4E5, E6*IE7E1^ΔE4E5 and E6*IIIE7E1^ΔE4E5 transcripts.

Table 2. Detection of HPV 16 transcripts in relation to the physical state of viral DNA in SCCs.

ISH	Physical state	E6/E7	Transcripts		
			E6*/I/II	E1 ^Δ E4 ^Δ L1	E1 ^Δ E4E5 ^a
Neg (n=2)	100%	100%	50%	0%	
I (n=50)	100%	98%	34%	54%	
I/E (n=64)	100%	98%	67%	95%	

ISH = *in situ* hybridization, I = Integrated, I/E = Integrated + Episomal. Neg = Negative by ISH. ^a The E1^ΔE4E5 primer-set also detect the E6E7E1^ΔE4E5, E6*IE7E1^ΔE4E5 and E6*IIIE7E1^ΔE4E5 transcripts.

Figure 1. Localization of NASBA primers and probes.

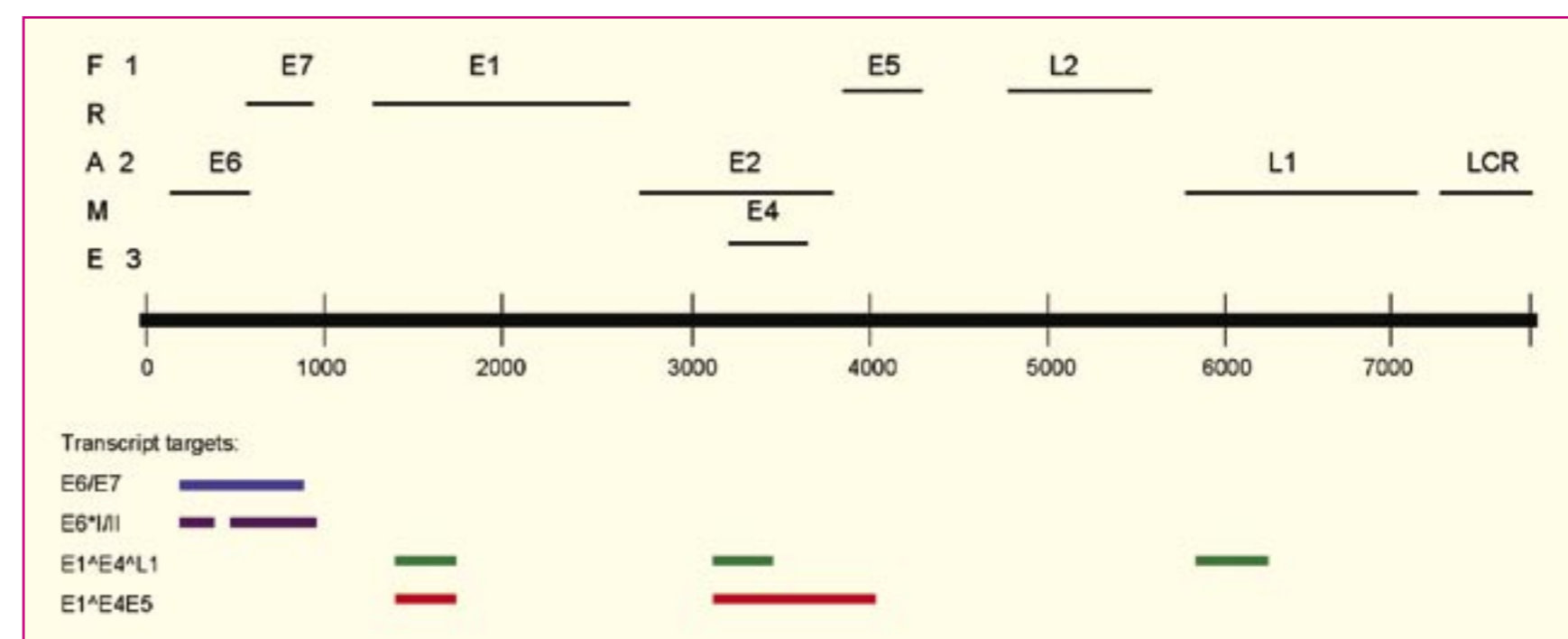
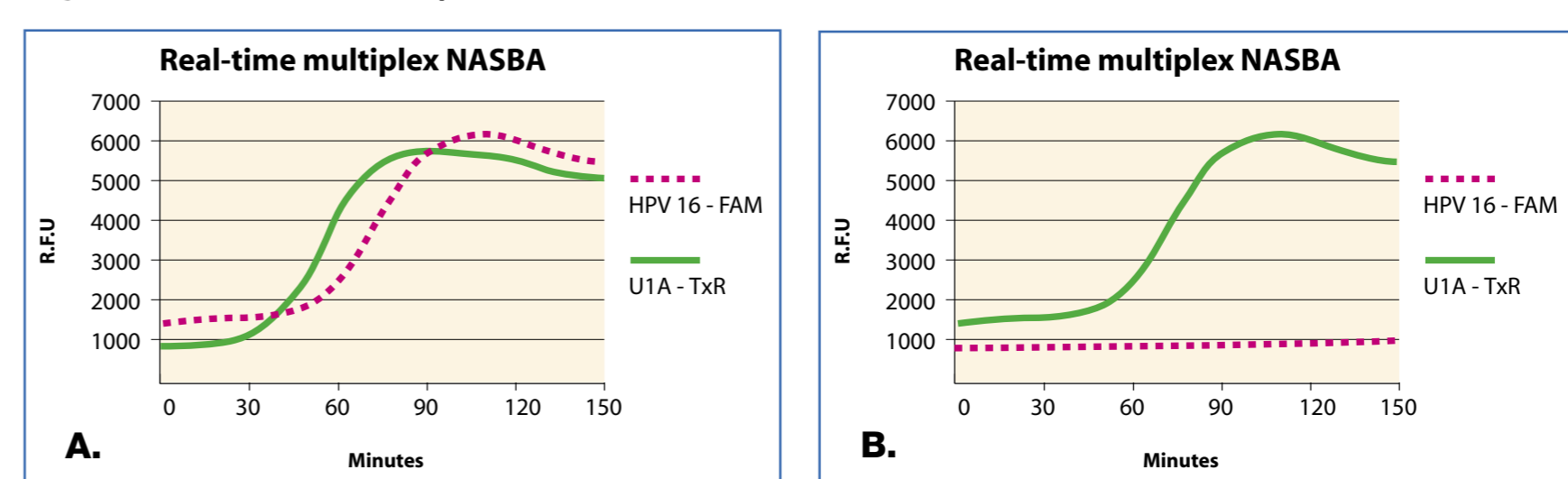


Figure 2. Real-time multiplex NASBA



A. Sample positive for HPV 16 mRNA and for the human U1A mRNA internal sample control.
B. Sample negative for HPV 16 mRNA but positive for the human U1A mRNA internal sample control.

Figure 3. Principle of NASBA

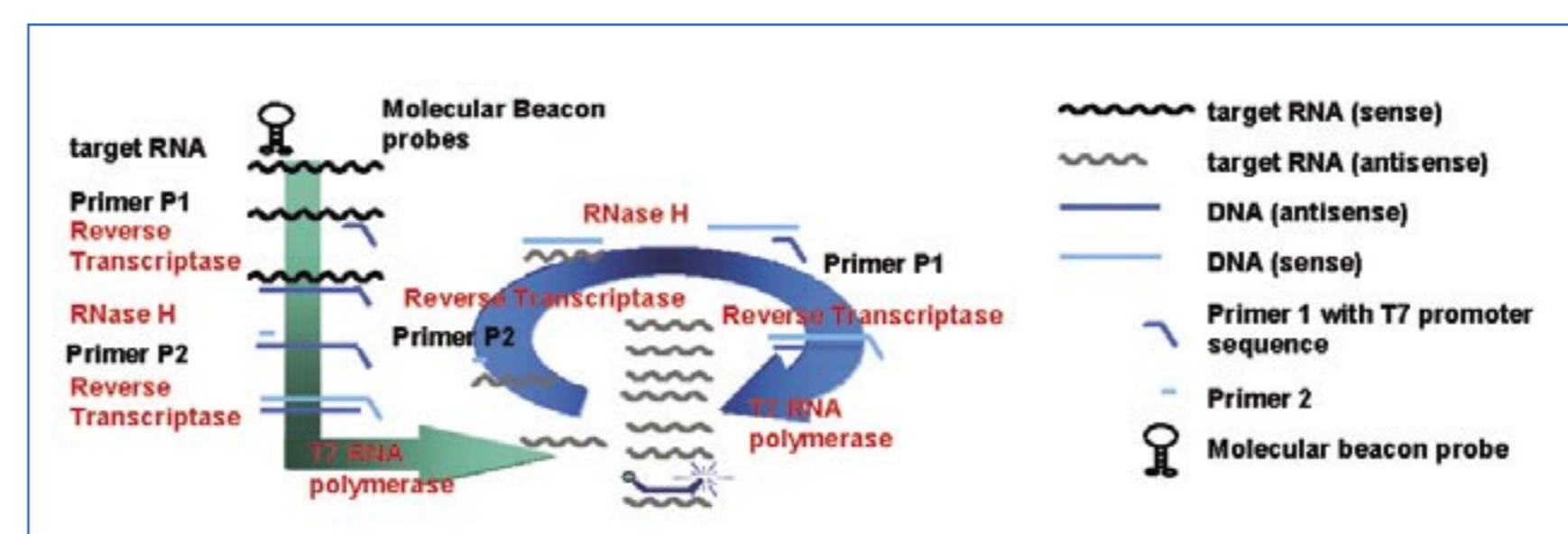
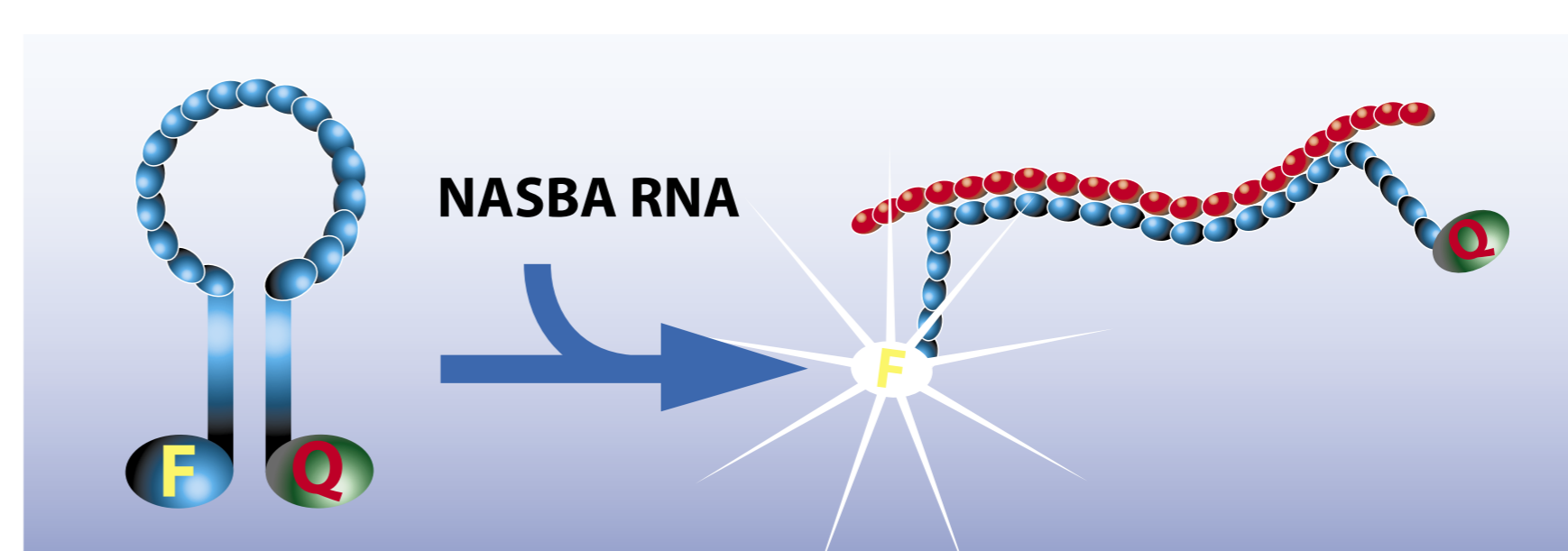
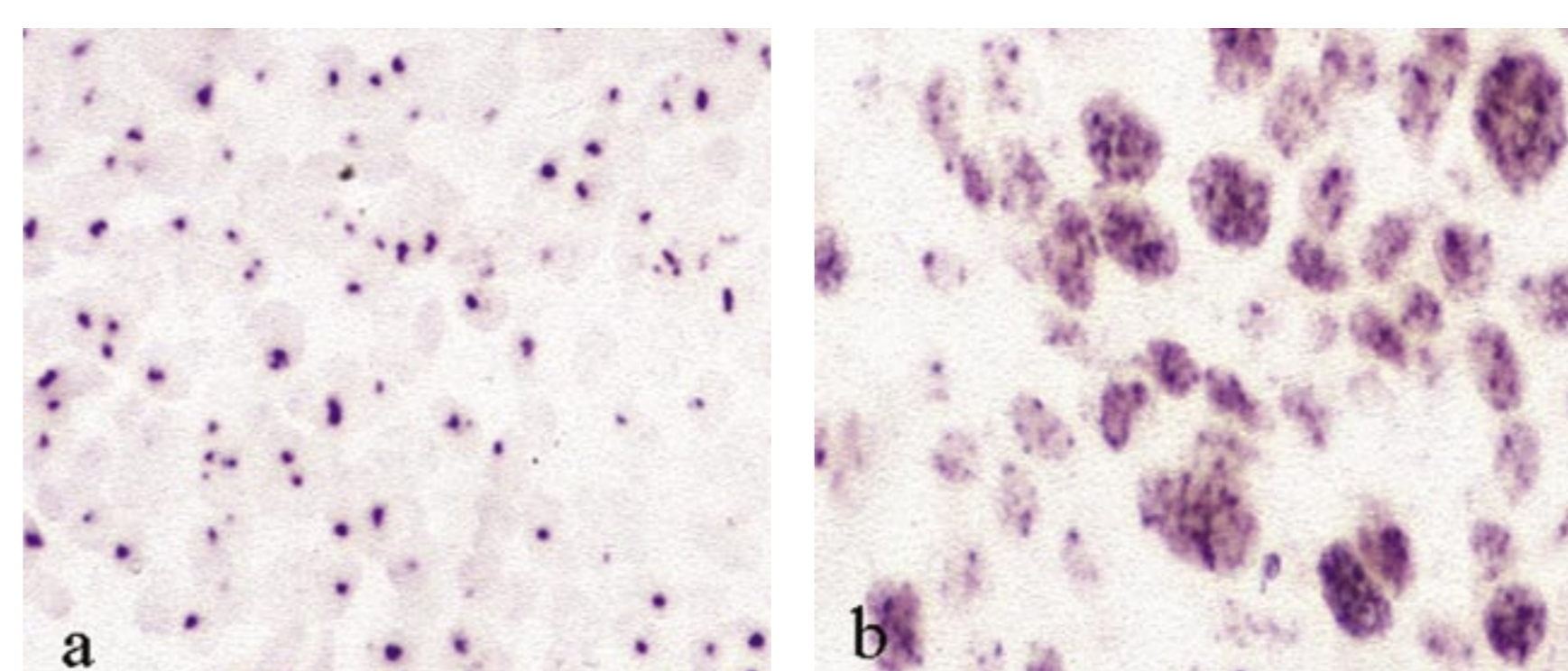


Figure 4. Principle of Molecular Beacon



Molecular Beacons are DNA probes with modified ends. In the folded state (stem-loop) the fluorophore is quenched, but upon binding of the loop sequence to its complementary target sequence, the probe undergoes a conformational change and a fluorescence signal is emitted. The probes will hybridize to the anti-sense RNA transcripts that are produced during the transcriptional phase of the NASBA reaction. While amplification proceeds, fluorescent signals are measured real-time in a fluorescent reader.

Figure 5. Positive HPV 16 signals by ISH



Punctuate ISH signal, indicating integrated HPV (a) and a punctuate and diffuse signal, indicating the presence of both integrated and episomal HPV (b).